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INTEGRATING BIODIVERSITY AND LANDSCAPE ECOSYSTEM PROCESSES: TESTS WITH FRESHWATER MUSSELS

A DISSERTATION APPROVED FOR THE
DEPARTMENT OF ZOOLOGY

BY

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Abstract

As humans alter the environmental landscape, ecosystems become increasingly imperiled due to habitat alteration and the associated species extinctions and extirpations. Consequently, recent research has often focused on how human altered landscape processes influence the distribution of species and the structure of biological communities. Further, recent research has also addressed how biodiversity itself is an important cog in the performance of ecosystem processes by biological communities. While both fields of research have yielded many important insights, they have both been limited by their scope. For example, research into how landscape processes influences species distributions and overall biodiversity often fail to recognize that biodiversity itself has the potential to feedback and influence landscape processes. Further, research into how biodiversity influences ecosystem function are often conducted on trophic processes within a single habitat, and fail to acknowledge that biodiversity might affect the physical transport of materials and resources across landscapes. My dissertation research aims to merge these areas of research to better integrate biodiversity into landscape ecosystem processes.

In my first chapter, I examine how high flow events in rivers, an important landscape process modified by humans via regulated releases by dams, influences the biodiversity of mussel communities. I sampled mussels and measured sediment and hydraulic variables (at low and high flows) at sites on the Little River, Oklahoma. To test which variables were most limiting mussel species richness, I evaluated univariate and multivariate 95^{th}-, 90^{th}-, and 85^{th}-quantile regression models using an information theoretic model selection approach. I found that models using hydraulic variables

related to substrate stability at high flows most limited mussel species richness. Models using the same variables estimated at low flows performed poorly. These results demonstrate that substrate stability at high flows is an important factor governing mussel distributions.

In my second chapter, I take the inverse view of my first chapter to test how mussel biodiversity itself influences substrate stability. I conducted a flume experiment, manipulating mussel species richness in a classic "biodiversity and ecosystem-function" design. Using three mussel species, I measured how species by themselves ("monocultures") influenced erosion of the streambed, and compared their performance to those of species mixtures ("polycultures" of 2 and 3 species). Mussel species vary in traits that should modify their effects on substrate erosion, such as shell morphology and burrowing behavior. Further, I crossed these mussel species treatments with two density treatments (high and low). I found that mussel species richness was associated with an increase in gravel erosion at both low and high densities. Planned contrasts showed that the erosion observed in species mixtures was purely additive at low density, as erosion in a polyculture could be routinely predicted by monoculture performance. However, at high density certain combinations of species showed non-additive effects on erosion, suggesting that organism abundance can fundamentally alter biodiversity effects. Further, this experiment shows that biodiversity can modify the physical transport of materials across landscapes.

In my third chapter, I investigate how mussel biodiversity can increase the flow of resources from aquatic to terrestrial ecosystems via a complex trophic cascade. Mussel biodiversity increases algae production in streams, which is followed by

increases in abundance of grazing aquatic insect larvae. Because aquatic insects are an important prey subsidy to terrestrial predators, I conducted experiments to see if mussel biodiversity increases the flux of aquatic insect prey subsidies to terrestrial predators. In a mesocosm experiment I found that mussel species richness was associated with an increase in algae production rates, aquatic insect emergence rates, and spider standing crop biomass. Effects of mussel polycultures on algae production could be predicted additively from monocultures, and mussel effects were linked through stable isotope analyses to mussel-derived nitrogen subsidies. In contrast, certain mussel species mixtures had non-additive effects on insect emergence. Mussel polycultures were associated with a more evenly distributed algae community than mussel monocultures, and aquatic insect emergence rates were higher in these more mixed algal assemblages. Finally spider standing crop biomass weakly tracked increases in aquatic insect emergence. In a field study of mussel communities on 2 rivers we found that sites with greater mussel species richness had higher aquatic insect emergence rates. These results show that because food webs in adjacent ecosystems are linked, effects of biodiversity losses on ecosystem functioning are not limited to the ecosystem in which extinctions occur.

CHAPTER 1

Complex hydraulic and substrate variables limit freshwater mussel species richness and abundance

Abstract. I examined how substrate and complex hydraulic variables limit the distribution of freshwater mussels. I sampled mussels and measured substrate and hydraulic variables (at low and high flows) at 6 sites in the Little River, Oklahoma. To test which variables were most limiting to mussel species richness and abundance, I evaluated univariate and multiple 95th-, 90th-, and 85th-quantile regression models using a model selection approach. Across all 3 quantiles, models using hydraulic variables related to substrate stability (relative shear stress ratio [RSS] and shear stress) at high flows most limited mussel species richness and abundance. High-flow substrate stability models performed the best, but models that used substrate variables (substrate size and heterogeneity) also performed relatively well. Models that used complex hydraulic variables estimated at low flows performed poorly compared to those using the same variables estimated at high flows, a result suggesting that hydraulic conditions at low flows do not limit mussel habitat in our system. My results demonstrate that substrate stability at high flows is an important factor governing mussel distributions. Finally, my quantile regression approach successfully quantified the limiting-factor relationships of substrate and hydraulic characteristics on mussel habitat, and this approach could be used in other studies investigating habitat requirements of aquatic organisms.

INTRODUCTION

Recent catastrophic declines in the abundance and diversity of freshwater mussel populations (Bivalvia:Unionoida) have led conservationists to recognize these animals as North America's most imperiled fauna (Strayer et al. 2004). Only ¼ of the ~300 North American species are considered to have stable populations (Williams et

al. 1993). Mussel population declines have multiple causes, including invasive species, water-quality degradation, and habitat alteration by impoundments (Lydeard et al. 2004, Strayer et al. 2004). Alteration of flow regimes by impoundments, channelization, and other man-made modifications has led to biodiversity losses in many riverine faunal groups (Poff et al. 2007), but freshwater mussel communities seem particularly sensitive to changes in hydrologic conditions (Watters 2000, Strayer et al. 2004).

Freshwater mussels often occur in dense multispecies aggregations (mussel beds) that are patchily distributed within streams and rivers. Locations of these aggregations and mussel abundance at smaller scales have been predicted successfully with complex hydraulic variables (Gangloff and Feminella 2007, Steuer et al. 2008, Zigler et al. 2008). Complex hydraulic variables related to near-bed flow characteristics, such as shear stress, are thought to be important factors for mussel habitat. Excessive shear stresses (hydraulic forces parallel to the substrate surface) at high flows can initiate substrate movement, so mussel aggregations are most likely to persist in areas where shear stresses remain low during spates, i.e., where substrates are stable (Strayer 1999, 2008, Strayer et al. 2004). Excessive shear stresses can also prevent juvenile mussels from settling into streambed substrates (Layzer and Madison 1995, Hardison and Layzer 2001), and mussel abundances are low in areas of high shear stresses during high flows (Hardison and Layzer 2001, Howard and Cuffey 2003, Gangloff and Feminella 2007).

However, several issues prevent mollusk ecologists from reaching a consensus on the importance of substrate stability for freshwater mussel distributions. First, shear

stress is not the only factor that influences substrate stability. Armoring and substrate size also are important factors determining whether substrates will become entrained during high flows (Gordon et al. 2004), so substrate characteristics must also be quantified. Studies that have used substrate characteristics and hydraulic variables have had some success predicting mussel abundance (Steuer et al. 2008). Second, very few studies have estimated hydraulic variables with data collected at both low and high flows (but see Hardison and Layzer 2001). Several authors have suggested that hydraulic variables should be more important at high than at low flows (Hardison and Layzer 2001, Howard and Cuffey 2003, Gangloff and Feminella 2007), but variables at high flows often are estimated from measurements of channel geomorphology rather than measured directly. Last, studies that have found substrate stability to be important for mussel habitat have primarily used computer simulations that have not been adequately ground-truthed. For example, the mussel dynamics model developed by Morales et al. (2006a) simulated mussel colonization using substrate stability to determine suitable habitats. However, this model has yet to be rigorously tested in the field and relies on many untested assumptions and parameter values (Morales et al. 2006b). Shear stress and substrate stability successfully predicted mussel abundance in a computer simulation by Zigler et al. (2008), but interpretation of these results was limited because of a significant time lag between the dates of collection of mussel and hydrologic data. The most rigorous support for substrate stability being an important factor for freshwater mussel habitat is from a field study in which mussels were most abundant in areas where marked stones moved the least during a spate (Strayer 1999). However, an alternative explanation for this result is that mussels themselves were

stabilizing substrates, such that the marked stones moved the least in areas where mussel abundances were highest. It has been suggested that freshwater mussels might stabilize substrates (Johnson and Brown 2000, Vaughn and Spooner 2006, Strayer 2008), although the results of a recent laboratory investigation were inconclusive (Zimmerman and de Szalay 2007).

Use of mussel abundance as the sole indicator of mussel habitat quality has limited our ability to interpret the results of studies on the relationships between freshwater mussel distributions and complex hydraulic variables (but see Gangloff and Feminella 2007). A positive relationship between substrate stability and mussel abundance is expected because when substrates are more stable over time, adult mussels should be less likely to be washed out during floods and the number of colonizing juvenile mussels surviving into adulthood should increase (Strayer 1999, Hardison and Layzer 2001, Hastie et al. 2001). However, different freshwater mussel species might prefer different hydraulic conditions or different levels of substrate stability. Although it seems likely that if substrate stability is associated with lower adult mussel mortality and greater juvenile mussel colonization more mussel species would also present, studies investigating relationships between substrate stability and mussel species richness are lacking. Given that declines in mussel species richness are as much of a concern as declines in mussel abundance (Lydeard et al. 2004, Strayer et al. 2004), there is a great need for studies that investigate habitat requirements for species rich mussel beds.

Most previous studies have used predictive statistical models to analyze relationships between complex hydraulic variables and mussel habitat quality

(Gangloff and Feminella 2007, Steuer et al. 2008, Zigler et al. 2008). Strayer (2008) argued that many factors in addition to hydraulic and substrate characteristics influence freshwater mussel distributions. These other factors include fish host distributions, food quality and quantity, water quality, and temperature. Therefore, even if substrate and hydraulic conditions are optimal, overall mussel habitat quality could be quite poor if these other requirements were not met (e.g. fish hosts not abundant or food quality low). Consequently, substrate and hydraulic variables should be analyzed as constraints or limiting factors rather than predictive variables because, at best, they can only partially explain mussel distributions.

I investigated how substrate stability (assessed with substrate and complex hydraulic variables) limits mussel habitat. I measured mussel species richness and abundance, substrate characteristics, and hydraulic variables in situ; and evaluated quantile regression models to determine whether and how these factors constrained mussel distributions.

METHODS

Study area and variables: I conducted the study in the Little River in southeastern Oklahoma, USA (Fig. 1). The Little River is a major tributary of the Red River that drains 10,720 km^2 in Oklahoma and Arkansas (Matthews et al. 2005). This river has high biodiversity and supports 110 fish and > 36 mussel species (Matthews et al. 2005). Mussel communities in this river have been studied (Vaughn and Taylor 1999, Galbraith et al. 2008), so I selected *a priori* 6 sites known to have abundant, diverse, and reproducing mussel communities (Fig. 1). Some mussel assemblages in the Little River are influenced by 1 mainstem and 1 tributary impoundment on the

Mountain Fork River (Fig. 1; Vaughn and Taylor 1999, Matthews et al. 2005). The tributary impoundment affects mussel communities primarily through cold-water releases and hydroelectric peaking, but all of our study sites were upstream of this influence. The mainstem impoundment (Pine Creek Reservoir) is used for flood control and recreation, but the influence of this reservoir is negligible downstream of the confluence with a tributary, the Glover River, which enters the Little River and modulates flows (Vaughn and Taylor 1999). My 6 sites were all downstream of the confluence of the Glover with the Little River and were only minimally affected by Pine Creek Reservoir, as evidenced by low summer flows (mean ± SE discharge during low-flow sampling = 0.63 ± 0.08 m^3/s), warm summer temperatures (mean = 30.6°C), and diverse and abundant mussel assemblages with juvenile recruitment. Seasonal median discharges calculated from monthly averages during 1977 to 2007 at a US Geological Survey (USGS) gauging station (07338500) immediately downstream of site 4 (Fig. 1) were 63.7 m^3/s in spring (March–May), 7.9 m^3/s in summer (June–August), 11.9 m^3/s in autumn (September–November), and 60.3 m^3/s in winter (December–February).

During July 2006, a period of low flows, I established 6 equidistant transects across the river at each site. Transects were 10 to 20 m apart depending on the size of the mussel bed (mean width of our transects across the river = 21.9 ± 0.94 m) and covered both riffles and pools. I measured water depth and current velocity at the centers of 1-m cells along 1 transect at each site for discharge calculations. I placed four 0.25-m^2 quadrats, evenly spaced across the river cross-section, along each transect. This stratified-block design and distance markers along the riverbank allowed

me to locate each quadrat easily by boat at higher flows (see below).

At each quadrat, I measured water depth with a meter stick and current velocity at 0.6 × depth with a Marsh–McBirney™ Flo-Mate flowmeter (Marsh–McBirney, Frederick, Maryland). I chose a random point in each quadrat and used a trowel to collect superficial substrates until I filled a 0.72-L plastic bag (~20% of the superficial substrate in the quadrat). Larger rocks (approximately ≥ 63.5 mm) were kept in separate bags so that we had at least a 0.72 L sample to process after substrates greater than 63.5 mm were excluded from the sample (to remove the bias of larger particles on substrate variables, Church et al. 1987). I sampled for mussels in each quadrat as the last step of the field protocol. I excavated each quadrat to a depth of 15 cm, removed all mussels from the quadrat, identified them, measured their shell length, and returned them to the quadrat (Vaughn and Spooner 2006, Galbraith et al. 2008). I took the substrate samples to the laboratory, dried them for 48 h at 100°C, passed the samples through a series of 12 geological sieves (63.5, 38.1, 19, 8, 3.962, 1.981, 0.991, 0.495, 0.246, 0.175, 0.088, and 0.061 mm), and weighed each fraction.

I returned to each site during periods of high flow between autumn 2006 and spring 2007 (mean discharge during high-flow sampling = 53.07 ± 7.92 m^3/s). I measured water depth and current velocity at the centers of 1-m cells along 1 transect at each site for discharge calculations, and I measured depth and current velocity at each quadrat on all 6 transects. I made all measurements from a boat secured to a cable stretched across the transect. We measured depth with a Hondex™ digital depth sounder (Honda Electronics Co. Ltd., Toyohashi City, Japan), and I measured current velocity by suspending a Marsh–McBirney™ Flo-Mate flowmeter fixed to a 13.6-kg

Columbus-type sounding weight (Scientific Instruments, Milwaukee, Wisconsin) on a marked cable at 0.6 × depth in the center of the quadrat.

I calculated substrate and hydraulic variables from formulae in Table 1. I refer to hydraulic variables estimated at low and high flows with LF and HF, respectively. I chose 0.065 as the value for Shield's parameter (θ_c) because substrates at our sites consisted of normally packed gravel with fairly random grain arrangements (Gordon et al. 2004). I calculated exceedance levels of our calculated discharge relative to historical data (1946–2007) from USGS gauging station 07338500 to quantify the relative flow levels represented by our data.

Data analysis

Multicollinearity among estimated hydraulic variables has been observed in other studies (Hardison and Layzer 2001). I wanted to reduce redundancy and multicollinearity bias ($r > 0.8$) in my statistical models because it can interfere with interpretation of results even though it would not violate the assumptions of our model selection approach described below (Burnham and Anderson 2002). I calculated Pearson correlation coefficients between all substrate and estimated hydraulic variables. Shear velocity (U_*) and shear stress (τ) were strongly correlated at low and high flows ($r = 0.87$ and 0.97, respectively), Froude number (Fr) and τ were strongly correlated at low and high flows (0.81 and 0.92), and HF boundary Reynolds number (Re_*) was strongly correlated with mean substrate particle size (D) ($r = 0.83$). Therefore, we dropped U_*, Fr, and Re_* from all subsequent analyses. I chose to keep τ and drop Fr and U_* because previous studies have shown relationships between τ and mussel distributions (Hardison and Layzer 2001, Howard and Cuffey 2003, Gangloff

and Feminella 2007) and because τ is important for substrate stability (Gordon et al. 2004). I chose to retain D instead of Re_* because substrate stability should be inversely related to substrate size given equal shear stresses (Gordon et al. 2004), and because Steuer et al. (2008) found that substrate size was a predictor of mussel abundance.

Quantile regression models have been used in ecological studies to estimate functions along or near the upper boundary of the response distribution to measure limiting factors (Cade and Noon 2003). Quantile regression is based on least absolute deviation regression, which models the conditional median (50^{th} quantile), but the approach can be extended to any quantile (Cade et al. 1999, Cade and Noon 2003, Koenker 2005). Quantile regression estimates are semiparametric, no parametric distributional form is assumed for random errors but is assumed for the deterministic portion of the model (Cade and Noon 2003). Therefore, unlike traditional least-squares regression, quantile regression relaxes the assumptions of normally distributed data and homoscedacity (Hao and Naiman 2007). In ecological studies, 95^{th}-quantile regressions have been used to estimate limiting-factor relationships; i.e. ~95% of the observations are below the fitted line (Schooley and Weins 2005).

One limitation of focusing on the 95^{th} quantile is that a large sample size is required for the analysis to be robust because a small fraction of the data (in this case, ~5%) is heavily weighted when parameter estimates are generated for regression functions and model fit is calculated. Our sample size was relatively small (n = 144), so I modeled 3 extreme quantiles (95^{th}, 90^{th}, 85^{th}) for a more robust analysis. I $\sqrt{x+1}$ transformed mussel abundance data before analysis (Zar 1999). I fit linear, quadratic,

or Ricker curves to the data (with and without *y*-intercepts), and chose the best-fitting model based on Akaike information criterion (AIC) provided it had non-0 parameter estimates for model coefficients. I used the same functions to fit multivariate quantile regression models. I conducted quantile regression analyses using the quantreg package (version 4.24 developed by R. Koenker) for R software (version 2.8.1; R Foundation for Statistical Computing, Vienna, Austria).

I evaluated quantile regression models with AIC. I followed Schooley and Wiens (2005) and calculated AIC for quantile regression models as $\mathrm{AIC} = n(\log \hat{\sigma}^2) + 2K$ (Hurvich and Tsai 1990), where K is the number of estimated variables + 2 (intercept and residual variance) and substituted the weighted absolute deviations (the absolute deviation of values predicted by the model from observed values, weighted by p for the p^{th} quantile if the predicted value > observed value and $[1 - p]$ if the predicted value < observed value; Hao and Naiman 2007) for $\hat{\sigma}$. I converted AIC to small-sample AIC ($\mathrm{AIC_c}$; Burnham and Anderson 2002), and calculated the coefficient of determination ® as 1 – (sum of the weighted absolute deviations of the model of interest divided by sum of the weighted absolute deviations of the intercept-only model) (Schooley and Weins 2005, Hao and Naiman 2007). I report a pseudo-R^2 for quantile regression models as $1 - (1 - R)^2$ to provide a unit of measure comparable to R^2 (McKean and Sievers 1987, Schooley and Wiens 2005).

I generated 15 models, 7 multivariate models and 8 univariate models a priori to avoid data-dredging and to ease interpretation of results (Johnson and Omland 2004). I chose the 7 multivariate models to represent different hypotheses that might explain mussel distributions: 1) substrate model (D + substrate heterogeneity [D

S.D.]), 2 and 3) LF and HF hydraulics models (LF or HF Re + τ + RSS), 4 and 5) LF and HF hydraulics and substrate models (LF or HF Re + τ + RSS + D + D S.D.), 6) HF substrate stability model (HF τ + RSS), and 7) a global model (all substrate and flow variables, an overparameterized model used for comparison). For each quantile, we report AIC_c differences (Δ_i) and Akaike weights (w_i, the relative likelihood of a model given a data set and set of models) for the 5 best models and the pseudo-R^2 of an averaged model based on predicted values from the best-performing models (Δ_i < 2) weighted by w_i (Burnham and Anderson 2002). Last, I determined the 5 best models across the 95th, 90th, and 85th quantiles by averaging w_i for each model from all 3 quantile model selection analyses.

RESULTS

Mussel communities were diverse and abundant at all 6 sites. Mean mussel species richness at our sites was 18.33 ± 0.76 (SE), and mean mussel abundance/m^2 was 44.95 ± 4.80. Juvenile mussels (individuals < 30 mm in length) were recorded at all sites. For more detailed descriptions of the mussel communities in the Little River, see Vaughn and Taylor (1999) and Galbraith et al. (2008). Low and high flow levels corresponded to exceedances of 95.15 ± 0.99 and 27.02 ± 2.06, respectively. Safety concerns prevented me from recording depth and flow measurements at peak flow levels (311.49 m^3/s was the highest recorded discharge at the USGS gauging station near my sites between 2006–2007).

Substrate and hydraulic variables estimated at low and high flows showed limiting-factor relationships with mussel species richness and abundance (Figs 2A–H, 3A–H). The limiting-factor relationships between *D* and mussel species richness and

abundance were unimodal and best described by the Ricker function for all extreme quantiles (Figs 2A, 3A). In contrast, the shape of the limiting-factor relationships between *D* S.D. and species richness and abundance differed depending on the quantile (Figs 2B, 3B). The limiting-factor relationships between Re and τ and species richness and abundance were unimodal and described by Ricker and quadratic functions at both low and high flows (Figs 2C–F, 3C–F). However, the shape of the limiting-factor relationships between RSS and species richness and abundance depended on flow level. The limiting-factor relationships between LF RSS and species richness and abundance were unimodal and described by the Ricker function (Figs 2G, 3G), whereas the limiting-factor relationships between HF RSS and species richness and abundance were decreasing functions. For species richness, the negative constraint was described by a linear function (Fig. 2H), whereas for abundance, it was described by the decreasing portion of a concave quadratic function (Fig. 3H).

Models with substrate variables performed best for the 95th quantile, whereas models with hydraulic variables related to substrate stability performed best for the 90th and 85th quantiles (AIC_c selection; Tables 2, 3). When Akaike weights (w_i) were averaged from our 3 quantile model selection analyses, models using hydraulic variables estimated at high flows performed better than models using substrate variables (Table 4). Summed average w_i for models with HF hydraulic variables were 0.79 and 0.61 for species richness and abundance, respectively, whereas summed average w_i for models with substrate variables for were 0.23 and 0.33 for species richness and abundance, respectively. HF RSS appeared to be the most important HF hydraulic variable because it was included in all of the best-performing models with

HF variables for both species richness and abundance. HF τ was important only in models that also included HF RSS for both species richness and abundance. HF Re was important only in models that included both HF RSS and HF τ, and only for species richness. Among models with substrate variables, summed average w_i for models with *D* were 0.23 and 0.17 for species richness and abundance, whereas summed average w_i for models with *D* SD were 0.18 and 0.31 for species richness and abundance, respectively. Models with LF hydraulic variables performed poorly (summed average w_i = 0.002 and 0.09 for species richness and abundance, respectively).

DISCUSSION

The most important result of this study was that across all 3 extreme quantiles analyzed, hydraulic variables related to substrate stability at high flows were most limiting for mussel species richness and abundance. Substrate models also performed well in the AIC_c selection analysis, but only at more extreme quantiles (95^{th} for species richness and abundance, and 90^{th} for abundance). Second, models with hydraulic variables estimated at high flows performed much better than models with the same variables estimated at low flows. Last, quantile regression is a useful analytical tool for investigating the ability of any single group of habitat factors to explain mussel distributions.

Hydraulic variables describing substrate stability at high flows were most limiting to freshwater mussel abundance and species richness. HF RSS alone or in conjunction with HF τ performed very well for both species richness and abundance. HF Re appeared to be less important, and only performed well in conjunction with HF

RSS and HF τ. These results support those of other studies suggesting that substrate stability during high flows restricts mussel abundance (Strayer 1999, Morales et al. 2006a, Gangloff and Feminella 2007). Moreover, my analysis is the first to show that substrate stability during high flows also restricts mussel species richness. Therefore, substrate stability during spates is likely to limit the distribution of dense and speciose mussel beds. My analysis also suggests that τ might not always be a useful surrogate measure for substrate stability, even when estimated at high flows. By itself, HF τ performed poorly in my analysis, and performed well only in the presence of HF RSS. This result shows the importance of quantifying both substrate characteristics and hydraulic variables to estimate substrate movement when assessing suitability of mussel habitat.

My estimates of substrate stability at high flows suggest that mussels might be able to tolerate some substrate movement. Mussel abundance and mussel species richness were high when HF RSS was > 1, but dropped sharply when HF RSS was > 2 (RSS > 1 indicates substrate movement, Figs 2H, 3H). However, our estimates of RSS used a typical sized particle (*D*50) to estimate substrate movement. Therefore, RSS > 1 does not necessarily mean that the entire streambed is in motion because *D*50 could represent just a small fraction of the larger materials sampled from the bed surface (Gordon et al. 2004). Thus, mussels might be able to tolerate movement of smaller substrate particles during high flows, but not movement of larger particles or the entire streambed. Furthermore, I omitted substrate particles > 63.5 mm from my substrate analysis to reduce the bias larger particles can have on substrate variables (Church et al. 1987). Omitting the largest substrate particles reduces *D*50 values and could have

caused overestimation of substrate movement. Alternatively, if mussels themselves stabilize substrates as other authors have suggested (Johnson and Brown 2000, Vaughn and Spooner 2006, Strayer 2008), all substrates might have remained stable at RSS > 1. Mussels increase sediment compaction and cohesion (Zimmerman and de Szalay 2007), which should decrease the ability of substrate particles to become entrained (Gordon et al. 2004). Estimates of substrate stability based on RSS use substrate and hydraulic variables, so biological influences on substrate stability are not taken into account. I think an in-depth study of the influence of mussels on substrate stability is warranted.

Models with the substrate variables *D* and *D* S.D. performed the best in 95th quantile regressions for both mussel species richness and abundance, but did not perform as well for any other quantile. This result might suggest that model performance was strongly influenced by data points at the boundary of the response distribution. Further evidence of this possibility is given by the differences among the best-fitting functions of the quantile regressions for *D* S.D. across the 95th, 90th, and 85th quantiles. For both species richness and abundance, the best-fitting quantile regressions for *D* S.D. did not have consistent mathematical functions. Instead the functions were linear, concave, or convex depending on the quantile (Figs. 2B, 3B). However, substrate variables were not entirely absent from models that performed well for quantiles other than the 95th, as the *D* S.D. model had $w_i = 0.163$ for the 90th quantile of mussel abundance. Therefore, substrate model performance might be somewhat spurious for the 95th quantile, but I think that substrate variables probably have a small limiting effect that is overwhelmed by HF hydraulic variables related to

substrate stability in our system. This disparity in the size of the effects might explain why substrate variables were important factors for mussel habitat in some studies (Steuer et al. 2008) but not in others (Strayer 1999).

Hydraulic variables estimated at high flows outperformed the same variables estimated at low flows. This result supports my hypothesis that hydraulic characteristics are more important to mussel habitat at high than at low flows, a conclusion that has been suggested by other authors (Hardison and Layzer 2001, Howard and Cuffey 2003, Gangloff and Feminella 2007). However, my results contrast with those of Steuer et al. (2008), who found that hydraulic variables estimated at low flows were better predictors of mussel abundance in the Upper Mississippi River than hydraulic variables estimated at high flows. Steuer et al. (2008) suggested that minimum $Re*$ and Fr might be required during low flows to deliver food or transport waste products. Thus, hydraulic variables estimated at low flows might not limit mussel distributions in smaller rivers, such as our system, but might be important in larger rivers, such as the Upper Mississippi River.

Quantile regression was a useful tool for studying the limiting effect of substrate and complex hydraulic variables on mussel species richness and abundance. The prevailing view in freshwater mussel ecology is that many factors in addition to hydraulic and substrate variables influence freshwater mussel distributions, including fish host distributions, food quantity and quality, and water quality (Strayer 2008). Thus, we should not expect any single group of variables to predict mussel habitat quality adequately. Rather, these variables should have limiting-factor relationships that constrain mussel distributions. For example, in my study the highest mussel

abundances and species richness occurred in quadrats with low HF RSS, but mussel abundance and species richness in other quadrats were low when HF RSS values were low (Figs. 2H, 3H). Presumably, some unmeasured factor was limiting in quadrats we estimated to be stable at high flows but with low mussel abundances or species richness.

We were able to quantify limiting-factor relationships with quantile regression models, in cases where predictive models would have had very low power. For example, the predictive power of substrate size, water depth, and water velocity on mussel abundance was very low ($r^2 < 0.05$) in a study by Strayer (1999), but a reanalysis with quantile regression of the data shown in fig. 4 in Strayer (1999) would be interesting and might show unimodal limiting-factor relationships. Quantile regression has the additional benefit that it relaxes the assumptions of normally distributed and homoscedastic data (Hao and Naiman 2007), and therefore, is very useful for analysis of ecological data (Cade and Noon 2003). Future studies investigating any single group of factors on mussel distributions should also use analyses that focus on quantifying limiting-factor relationships.

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TABLES

Table 1. Summary of substrate variables and hydraulic variables estimated at low and high flows. D_x = substrate particle size (cm) at which x% of the sample by mass is finer, d = water depth (cm), ϕ = unit of substrate size ($\phi = -\log_2 D$[mm]), ϕx = substrate particle size (ϕ) at which x% of the sample by mass is finer, U = mean current velocity (cm/s), g = acceleration due to gravity (980 cm/s), v = kinematic viscosity of water (0.01 cm^2/s), ρ = density of water (0.998 g/cm^3), ρ_s = density of substrate (2.65 g/cm^3), θ_c = Shield's parameter (0.065) (Gordon et al. 2004).

Variable (symbol, unit)	Formula	Description	Source
Substrate variables			
D (cm)	$\frac{(D_{16} + D_{50} + D_{84})}{3}$	Mean particle size of sample	Folk 1965
Sorting index (D S.D.; ϕ converted to cm)	$\frac{(\phi 84 - \phi 16)}{2}$	Substrate heterogeneity	Gordon et al. 2004
Bed roughness (k_s, cm)	$3.5 \times D_{84}$	Topographical variation of stream bed	Gordon et al. 2004
Hydraulic variables			
Froude number (Fr, dimensionless)	$\sqrt{\frac{U^2}{gd}}$	Ratio of inertial to gravitational forces	Statzner et al. 1988
Reynolds number (Re, dimensionless)	$\frac{Ud}{v}$	Ratio of inertial to viscous forces	Statzner et al. 1988
Boundary Reynolds number (Re., dimensionless)	$\frac{U_* k_s}{v}$	Roughness of flow near substrate	Statzner et al. 1988
Shear velocity (U., cm/s)	$\frac{U}{5.75 \log_{10}\left(\frac{12d}{k_s}\right)}$	Friction velocity	Statzner et al. 1988
Shear stress (τ, dynes/cm^2)	$\rho\left(U_*^2\right)$	Force of friction on substrate	Statzner et al. 1988
Critical shear stress (τ_c, dynes/cm^2)	$\theta_c g D_{50} \left(\rho_s - \rho\right)$	Shear stress required to initiate substrate motion for a typical sample substrate size (D_{50})	Gordon et al. 2004
Relative shear stress (RSS, dimensionless)	$\frac{\tau}{\tau_c}$	Ratio of observed to critical shear stress (values > 1 represent substrate movement for a typical sample substrate size [D_{50}])	Morales et al. 2006a

Table 2. Summary of small-sample Akaike information criterion (AIC_c) selection of univariate and multiple 95th, 90th, and 85th quantile regression models for mussel species richness. LF and HF designate that the model used hydraulic variables estimated at low or high flows. K = number of parameters in model + 2, $\Delta_i = AIC_c$ of model relative to lowest AIC_c, w_i = Akaike weight, R^2 = pseudo-R^2 of an averaged model using the best-performing models ($\Delta_i < 2$). Only the 5 best-performing models are shown, abbreviations for variables are given in Table 1.

Rank	Model	K	Δ_i	w_i
	95th quantile (R^2 = 0.22)			
1	D + D S.D.	6	0.000	0.472
2	HF RSS	3	1.142	0.267
3	HF Re + τ + RSS	7	2.427	0.140
4	D	4	3.705	0.074
5	HF τ + RSS	5	4.944	0.040
	90th quantile (R^2 = 0.14)			
1	HF τ + RSS	5	0.000	0.336
2	HF Re + τ + RSS	7	0.304	0.289
3	HF RSS	3	0.517	0.259
4	D + D S.D.	5	4.050	0.044
5	HF Re + τ + RSS + D + D S.D.	10	4.496	0.035
	85th quantile (R^2 = 0.17)			
1	HF RSS	3	0.000	0.371
2	HF τ + RSS	5	0.220	0.333
3	HF Re + τ + RSS	7	0.802	0.258
4	D + D S.D. + HF Re + τ + RSS	11	7.750	0.011
5	HF τ	4	7.897	0.007

Table 3. Summary of small-sample Akaike information criterion (AIC_c) selection of univariate and multiple 95th, 90th, and 85th quantile regression models for mussel abundance. LF and HF designate that the model used hydraulic variables estimated at low or high flows. K = number of parameters in model + 2, $\Delta_i = AIC_c$ of model relative to lowest AIC_c, w_i = Akaike weight, R^2 = pseudo-R^2 of an averaged model using the best-performing models ($\Delta_i < 2$). Only the 5 best-performing models are shown, abbreviations for variables are given in Table 1.

Rank	Model	K	Δ_i	w_i
	95th quantile (R^2 = 0.22)			
1	D + D S.D.	4	0.000	0.415
2	D S.D.	3	0.926	0.261
3	HF τ + RSS	6	1.860	0.164
4	LF Re	4	2.590	0.114
5	HF τ	4	5.797	0.023
	90th quantile (R^2 = 0.14)			
1	HF τ + RSS	6	0.000	0.356
2	HF RSS	4	0.188	0.324
3	D S.D.	4	1.563	0.163
4	LF Re	4	3.551	0.060
5	HF τ	4	4.010	0.048
	85th quantile (R^2 = 0.17)			
1	HF τ + RSS	6	0.000	0.351
2	HF RSS	4	0.691	0.249
3	HF Re + τ + RSS	8	1.024	0.211
4	HF τ	4	3.879	0.051
5	LF Re	4	4.184	0.043

Table 4. Akaike weights (w_i) averaged from small-sample Akaike information criterion (AIC_c) selection of univariate and multiple 95th, 90th, and 85th quantile regression models for mussel species richness and abundance. LF and HF designate that the model used hydraulic variables estimated at low or high flows. Models with 5 highest average Akaike weights (w_i) are shown, abbreviations for variables are given in Table 1.

Species richness		**Abundance**	
Model	*Average* w_i	*Model*	*Average* w_i
HF RSS	0.299	HF τ + RSS	0.290
HF τ + RSS	0.236	HF RSS	0.195
HF Re + τ + RSS	0.229	D S.D.	0.151
D + D S.D.	0.174	D + D S.D.	0.146
D	0.034	HF Re + τ + RSS	0.078

FIGURES

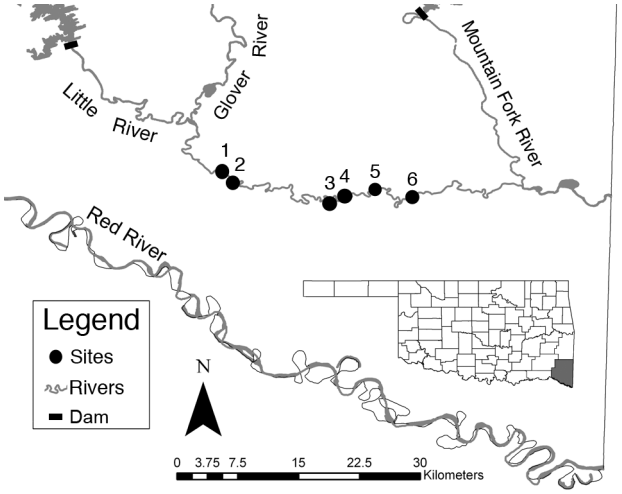
Figure Legends

Figure 1. Sampling sites on the Little River in southeastern Oklahoma for chapter 1.

Figure 2. Quantile regression models for mussel species richness/0.25-m^2 quadrat for substrate (A, B) and hydraulic (C–H) variables. Solid, dashed, and dotted lines represent 95^{th}, 90^{th}, and 85^{th} quantile regression lines, respectively. LF and HF designate that the variable was estimated at low or high flows. Abbreviations for variables are given in Table 1.

Figure 3. Quantile regression models for $\sqrt{\text{mussel density} + 1}/m^2$ for substrate (A, B) and hydraulic variables (C–H). Solid, dashed, and dotted lines represent 95^{th}, 90^{th}, and 85^{th} quantile regression lines, respectively. LF and HF designate that the variable was estimated at low or high flows. Abbreviations for variables are given in Table 1.

Figure 1.


Little River
Glover River
Mountain Fork River
Red River
1
2
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4
5
6
Legend
Sites
Rivers
Dam
N
0 3.75 7.5 15 22.5 30
Kilometers

Figure 2.

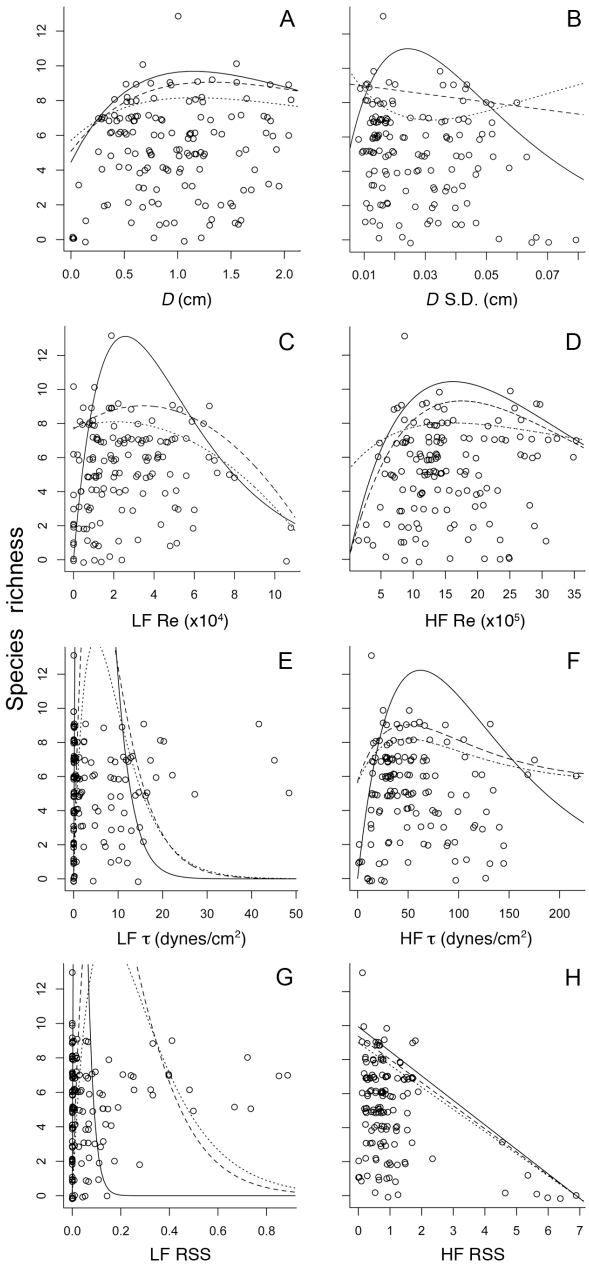
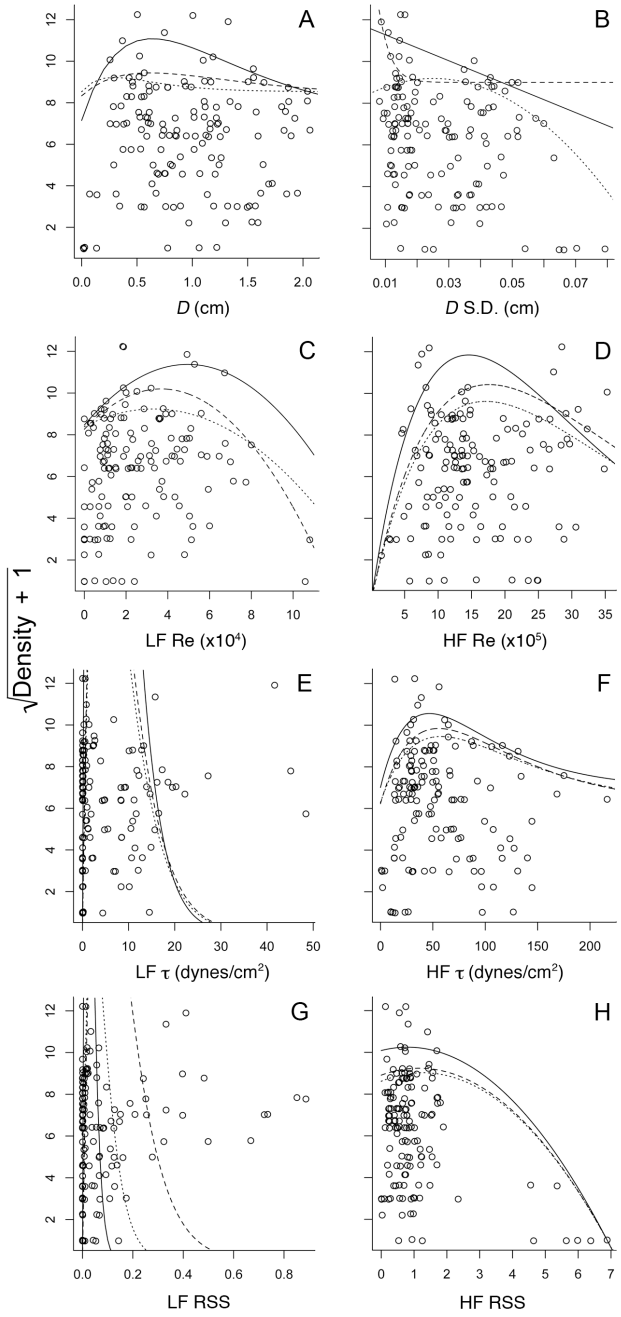

A
B
C
D
E
F
G
H
Species richness
D (cm)
D S.D. (cm)
LF Re (x10⁴)
HF Re (x10⁵)
LF τ (dynes/cm²)
HF τ (dynes/cm²)
LF RSS
HF RSS

Figure 3.


A
B
C
D
E
F
G
H
√Density + 1
D (cm)
D S.D. (cm)
LF Re (x10⁴)
HF Re (x10⁵)
LF τ (dynes/cm²)
HF τ (dynes/cm²)
LF RSS
HF RSS

CHAPTER 2

Density-dependent biodiversity effects on physical habitat modification by freshwater bivalves

Abstract: Several decades of research have shown that biodiversity affects ecosystem processes associated with resource capture and the production of biomass within trophic levels. Although there are good reasons to expect that biodiversity influences non-trophic ecosystem processes, such as the physical creation or modification of habitat, studies investigating the role of biodiversity on physical processes are scarce. Here we report the results of a study using artificial streams to test the influence of freshwater mussel biodiversity on gravel erosion during high flows while manipulating mussel abundance. Mussel species vary in traits that should influence their effects on erosion, such as size, shell morphology and burrowing behavior. We found that mussel species richness was associated with an increase in erosion at both low and high densities. Planned contrasts showed that the erosion observed in species mixtures was purely additive at low density, indicating that erosion in a species polyculture could routinely be predicted by the performance of monocultures. However, at high density certain combinations of species showed non-additive effects on erosion, suggesting that organism abundance can fundamentally alter biodiversity effects. Although this may have been a result of altered species interactions at high density, our study design cannot confirm this.

INTRODUCTION

The biodiversity of ecological communities can significantly affect the performance of ecosystem processes (Hooper et al. 2005). However, most BEF studies have focused on ecosystem processes related to resource capture or production of biomass within trophic levels, or the flow of energy and nutrients between them (Hooper et al. 2005). Biodiversity effects on trophic ecosystem processes such as

resource use or prey consumption are expected where niche partitioning has evolved due to competition for common resources. Nevertheless, we might also expect biodiversity effects on non-trophic ecosystem processes (such as creating and modifying habitat) due the diversity of physical structures produced by organisms, but studies investigating this are lacking.

Here I examine how species diversity of an important group of ecosystem engineers can impact the physical transport of sediment in stream ecosystems. Ecosystem engineers are organisms whose physical modifications to habitats have strong effects on other species and ecosystem processes (Jones et al. 1994). In streams, benthic organisms can physically modify habitats in ways that influence sediment transport during high flow events. Flood disturbances can regulate the diversity and function of benthic ecosystems across temporal and spatial scales (Resh et al. 1988). Species that stabilize sediments during high flows and prevent sediment entrainment, such as such as net-spinning caddisflies (Cardinale et al. 2004) and water willow (Fritz et al. 2004), can have significant effects on stream ecosystems. For example, Cardinale et al. (2004) estimated that net-spinning caddisflies could reduce the probability of a riverbed-scouring flood by 17%. Other stream species, such as some benthic fish and crayfish, can destabilize sediments during high flows (Statzner and Sagnes 2008). Species with stabilizing effects tend to bind sediment particles together through biological activity, while species with destabilizing effects tend to be bioturbators (mixers and disrupters of sediment through biological activity such as burrowing). Although some studies have investigated interactive effects of species on

substrate stabilization (Statzner and Sagnes 2008), studies investigating the role of community structure and diversity on sediment transport are lacking.

Mollusks function as ecosystem engineers in many habitats (Gutierrez et al. 2003). Freshwater mussels (Bivalvia: Unionidae, hereafter "mussels") are large, long-lived mollusks that can dominate benthic biomass in streams (Strayer 2008). Mussels are a globally imperiled fauna due to both species extinctions and declines in abundance of once common species (Strayer 2008). In streams, mussels typically occur as multispecies assemblages called "mussel beds" (Strayer 2008). Mussel species vary in multiple traits that should influence their ability affect sediment transport, such as size, shell morphology, and burrowing activity (Allen and Vaughn 2009). Active burrowing species should destabilize sediments through bioturbation, while sedentary species that burrow deeply should stabilize sediments by increasing compaction and cohesion (Allen and Vaughn 2009). Further, mussel size, shape and shell morphology should modify effects on substrate erosion (Watters 1994). Hydraulic principles suggest that large species with smooth shells exposed to flow will increase near-bed turbulence, destabilizing substrates (Vogel 1994). In contrast, species with textured shells should mitigate the increased turbulence patterns generated by exposed shells that could initiate erosion (Watters 1994). In addition, higher mussel species richness may increase the topographical complexity of the streambed, increasing near-bed turbulence similar to patterns observed with net-spinning caddisfly larvae (Cardinale et al. 2002).

I performed experiments examining the effects of mussel richness and abundance on sediment transport during high flows. I hypothesized that: 1) increasing

mussel species richness will increase sediment erosion because exposed mussels will increase the topographical complexity of the bed surface and may increase near-bed turbulence, 2) because the density of roughness elements is well known to control turbulence and shear, biodiversity effects are likely to differ at low and high densities, and 3) mussel species with sculptured shell morphologies should stabilize substrates, species that are active burrowers should destabilize substrates, and that these effects should increase with density.

METHODS

Studies investigating the effects of organisms on sediment transport are generally conducted in artificial channels, or "flumes", where factors influencing sediment transport (such as water velocity, water depth, and sediment composition) can be controlled (Vogel and LaBarbera 1978, Nowell and Jumars 1987, Cardinale et al. 2004). I constructed 8 re-circulating stream channels modeled after Vogel and LaBarbera (1978) to standardize hydrodynamic and sediment properties across our experimental treatments (Fig. 1). Each flume measured 330 cm x 38.1 cm x 33 cm, and contained a 33 cm x 33 cm working area with a 12.7 cm deep false bottom 264 cm from the flow entrance (8x the channel width as recommended by Nowell and Jumars [1987]). Each flume contained a 38.1 cm x 33 cm x 4.6 cm collimator constructed of 5.4 mm diameter plastic straws (recommended by S. Vogel, pers. 36meri.). Current velocity was manipulated with a ¾ hp speed-controlled motor with 2 propellers separated by a stator on the drive shaft. Some hydraulic aspects of my flumes (as described by Froude number, Reynolds number, boundary Reynolds number, and shear stress) scale to those observed in natural mussel beds (Allen and Vaughn 2010,

Table 1), and describe hydraulic conditions that are sub-critical, turbulent, and hydraulically rough near the bed-water interface.

Prior to each trial, gravel particles (mean diameter 4.84 mm ± 1.78 S.D., similar to those found in natural mussel beds [Allen and Vaughn 2010]) were homogenized, added to the working area in each stream channel, and gently compacted and leveled. In pilot studies, when mussels burrowed they displaced gravel above the lip of the working area, so that the gravel level was no longer flush with the flume bottom. Further, the amount of gravel displaced was proportional to the number and size of the buried mussels. Gravel displaced above the flume bottom would be exposed to additional forces of drag and lift since it would be more directly exposed to flow, which could introduce a bias in our experiment. To ensure that the gravel level remained constant for all mussel and no-mussel treatments, I standardized substrate volumes among treatments. I estimated the expected volume of gravel that would be displaced by the mussels using data from a previous study of mussel burrowing behavior. I measured the volume of mussels to be added, and then multiplied this value by the mean proportion of mussel body buried in sediment for that species as measured by Allen and Vaughn (2009). This calculation gave us the volume of gravel the mussels would displace from the working area while buried, which was then removed so that each treatment would have a gravel level flush with the bottom of the flume. Gravel particles were then gently compacted and leveled a second time, and the stream channels were filled with water to 33 cm depth. Mussels were added at random points using a grid with 16 equal sections, and were oriented in the gravel with the posterior end facing downstream and siphons facing upstream (a typical natural

orientation in streams). Mussels were acclimated at flow velocities of 5 cm/s and allowed to burrow for 4 days.

I used 3 mussel species that naturally co-occur in mussel beds in SE Oklahoma (with any one species being dominant in a given mussel bed), but that vary in traits that should influence their effects on sediment transport (Fig. 2). *Actinonaias ligamentina* is large (mean length of individuals used in this experiment was 105.2 mm), is an active epi-benthic burrower (burrows above the sediment-water interface [Allen and Vaughn 2009]), and has a smooth shell with no anchoring sculpture (ridges or pustules that help hold a mussel in place in substrates) or anti-scouring sculpture (ridges or pustules that disrupt scouring hydraulic forces [Watters 1994]). Amblema plicata is medium sized (mean length 83.9 mm), a sedentary epi-benthic burrower (Allen and Vaughn 2009), and has an anchoring sculpture and an anti-scouring sculpture along the posterior slope and dorsal ridge (Watters 1994). Quadrula pustulosa is a small (mean length 48.6 mm), sedentary endo-benthic burrower (burrows below the sediment-water interface [Allen and Vaughn 2009, unpublished data]), and has an anchoring sculpture and anti-scouring sculpture along the dorsal ridge (Watters 1994). These traits suggest that A. ligamentina should have destabilizing effects on sediments, while A. plicata and Q. pustulosa should have stabilizing effects. Mussels (A. ligamentina [n = 82], A. plicata [n = 84], and Q. pustulosa [n = 80]) were collected from a single site on the Kiamichi River in SE Oklahoma. Mussels were held in a Living Stream (Frigid Units Inc., Toledo, OH) for 2 weeks prior to the experiment and fed 500 mL of cultured algae per stream channel daily.

I manipulated mussel community structure with 2 density treatments crossed with 8 species composition treatments in a factorial design. The low and high-density treatments were 6 and 12 mussels per flume (corresponding to densities of 55 and 110 mussels/m^2), representing natural densities of mussels observed in rivers in SE Oklahoma (Allen and Vaughn 2010). The 8 species composition treatments were 3 "monocultures" (single species treatments), 4 "polycultures" (each possible 2-species combination and a 3-species combination), and a no-mussel control. Each species composition treatment was replicated 12 times at each density. Mussels were randomly assigned to treatments, and treatments were randomly assigned to flumes in each trial. A trial consisted of one density treatment per trial with density treatments randomly assigned to trials, for a total of 24 trials. On day 4 of each trial I measured water temperature, digitally photographed mussel burrowing positions, and measured width, length, and height of exposed shell for each mussel. These measurements and the digital image were used to generate a suite of burrowing variables (see below). Flow velocities were then increased to the maximum flow speed (~83 cm/s) for 2 minutes, which pilot studies showed was enough time for all substrate erosion to occur. Eroded gravel was caught in a 1 mm mesh net downstream of the working section (Fig. 1), dried for 48 hrs and weighed.

Statistical Analyses: I first analyzed the relationship between mussel species richness and gravel erosion at both density treatments using linear regressions on log-transformed raw weights (grams, g) of eroded gravel, with mussel species richness as the explanatory variable. To analyze the influence of mussel diversity and density treatments on substrate stability, I wanted a metric that was standardized relative to the

performance of the no-mussel controls. We subtracted the log-transformed mean value of eroded gravel (g) from no-mussel treatments from each datum of log-transformed eroded gravel (g) from stream channels with mussel treatments. This is essentially a log (x +1) – k transformation where x is each datum (raw weight of gravel eroded from a given flume) and k is a constant value (the log-transformed mean weight of gravel eroded from no-mussel control treatment flume runs). The resulting variable can be defined as increasing erosion relative to controls if the value was positive (or decreasing erosion if negative), which we will refer to as "net change in gravel erosion (g)." I then ran a mixed model 2-way ANOVA on the net change in gravel erosion with mussel density and species composition treatments as fixed effects, and with trial as a random effect to account for any temporal differences.

Following other BEF experiments (Douglass et al. 2008, Griffin et al. 2008), I conducted 16 a priori planned linear contrasts to test for non-additive biodiversity effects. The first set of contrasts (n = 8) tested the null hypothesis that the observed polyculture mean is the same as the expected mean based on additive monoculture performances (i.e. a two species polyculture treatment was given a contrast coefficient of 1, while the 2 monoculture treatments of the species present in that polyculture were given contrast coefficients of -0.5; testing the null hypothesis that the mean of the polyculture was equal to the weighted means of its monocultures). The second set of contrasts (n = 8) compared the observed polyculture performance against its monoculture with the strongest effect on gravel erosion. These two types of contrasts represent a liberal and a conservative test for non-additive biodiversity effects, respectively, and I refer to them as such.

Table-wide adjustments have been recommended to decrease the increased probability of Type I errors when performing multiple comparison procedures (Rice 1989), but recently others have criticized such methods for obscuring ecologically significant effects and increasing Type II errors (Nakagawa 2004). Therefore, I followed Douglass et al. (2008) and opted not to apply a table wide adjustment to *P*-values to our contrasts, but rather I report the effect size of each test along with exact *P*-values whenever possible, using both to interpret ecological significance (i.e. if the results of a contrast was statistically significant but had a small effect size, I would view the result with caution). Further, I interpret the results of multiple statistical tests strictly within the context of our broader hypotheses (i.e. whether or not biodiversity effects are present). Effect sizes are reported as the partial omega squared, ω^2, which measures the variability of the contrast relative to itself and the error, and is not influenced by the main treatment effects: $\omega^2_{\langle\psi\rangle} = \frac{\sigma^2_\psi}{\sigma^2_\psi + \sigma^2_{error}}$, which I estimated using the formula: $\omega^2_{\langle\psi\rangle} = \frac{F_\psi - 1}{F_\psi - 1 + 2n}$, where F_ψ is the *F*-statistic of the contrast and n is sample size (Keppel and Wickens 2004).

Burrowing variables: Burrowing activity of marine bivalves influences erosion (Sgro et al. 2005), and exposed bivalve shells increase near-bed turbulence and promote erosion in marine systems (Widdows et al. 2002). Because freshwater mussel species vary in burrowing activity and depth (Allen and Vaughn 2009), I wanted to see if mussel effects on gravel erosion were partly due to burrowing behavior. We calculated a suite of burrowing variables from measurements of exposed

mussel shells (width, depth, and height) and from measurements taken from digital photographs (distance between mussel pairs and mussel orientation relative to flow direction). Using these measurements, I calculated 9 burrowing variables that I thought might influence substrate stability: surface area added by mussels (cm2; mean, S.D., and sum), mussel orientation relative to flow direction (degrees; mean, S.D., and sum), and distance between mussel pairs (cm; mean, S.D., and number of pairs < 2.5 cm apart). The number of mussel pairs < 2.5 cm apart (an arbitrary distance chosen) was measured to estimate the clustering of mussels in an experimental run. I ran a stepwise multiple linear regression analysis on the net change in gravel erosion (g) to see which variables were most important.

RESULTS

Linear regressions showed significant increasing relationships between mussel species richness and gravel erosion at both low ($y = 1.704 + 0.088x$, $P = 0.002$, $R^2 = 0.08$) and high densities ($y = 1.66 + 0.070x$, $P = 0.045$, $R^2 = 0.04$; Fig. 3). The relatively low R^2 values of the linear regressions are partly due to differences between multiple species treatments within a single value of species richness, but also because of variation within treatments. The magnitude of the species richness effect was strong, as the mean gravel erosion in 3-species polycultures was 77.1% and 93.8% greater than that of no-mussel controls at low and high density, respectively.

The mixed model 2-way ANOVA showed a significant species treatment effect ($F_{6, 132} = 2.705$, $P = 0.016$), an insignificant density effect ($F_{1, 22} = 1.986$, $P = 0.173$), and an insignificant species composition X density interaction ($F_{6, 132} = 0.857$, $P = 0.528$) on the net change in gravel erosion (Fig. 4). All planned contrasts testing

for non-additive biodiversity effects were insignificant at low density. At high density 2 of 4 liberal contrasts testing for non-additive biodiversity effects were significant, and 1 of 4 conservative contrasts was significant (Table 2). The observed effect sizes of these contrasts fall within the range of those reported by another BEF study, Douglass et al. (2008). The magnitude of the non-additive biodiversity effects were quite large, as the high-density 3-species polyculture observed 51.9% more erosion than expected given additive performances of its monocultures, while the high-density Amblema plicata and Quadrula pustulosa polyculture observed 49.9% more erosion than additive expectations.

Stepwise multiple regression analysis of the net change in gravel erosion using the suite of burrowing variables produced two significant models (Table 3). The models indicate that the most important burrowing variable was "mussel orientation relative to flow direction", the only variable in Model 1, which explained approximately 11% of the variation in gravel erosion relative to controls. Model 2 added the burrowing variable SD of topographical surface area added by mussels, which increased the amount of variation explained to approximately 13%. None of the other 7 burrowing variables were included in significant multiple regression models.

DISCUSSION

Mussel species richness was associated with an increase in gravel erosion at high flows relative to controls at both low and high density, but the nature of this relationship differed between high and low density treatments. At low density, all planned contrasts testing for non-additive biodiversity effects were insignificant. This suggests that the performance of species polycultures on erosion could be routinely

additively predicted from the performance of species monocultures. However, at high density certain species polycultures had significant non-additive biodiversity effects on gravel erosion. This suggests that mussel abundance fundamentally altered the nature of biodiversity effects on gravel erosion. Mussel species traits that influence substrate erosion may be interacting at high densities and not at low densities, although our study design is unable to confirm this. Nevertheless, the results of our study support others that have found that organism abundance can modify the BEF relationship (Douglass et al. 2008, Griffin et al. 2008).

Mussel species treatments significantly differed in their effect on gravel erosion relative to no-mussel controls. Because freshwater mussels vary in burrowing behavior (Allen & Vaughn 2009), and because burrowing activity by bivalves in marine systems promotes erosion (Sgro et al. 2005), I hypothesized that burrowing behavior by mussels might be a plausible mechanism to explain mussel effects on gravel erosion. Although the multiple regression models using burrowing variables only explained 13% of the variation in gravel erosion, my analysis lends some support to this hypothesis. The burrowing variable "mussel orientation relative to flow direction" was the single best variable in multiple regression models (explaining 11% of variation on its own). There are 2 possible explanations for increases in substrate erosion when mussels are oriented in ways that deviate from the flow direction (when the mussel's anterior-posterior axis is not parallel to the flow direction). First, mussel species that are more active burrowers are more likely to move and deviate from their original position and disrupt cohesive properties of sediment in the process. Alternatively, when the orientation of a mussel deviates from the flow, it is less

hydrodynamic and generates larger wake patterns whose ascending vortices can promote erosion (Vogel 1994). Ultimately, the relatively low explanatory power of our burrowing models suggests that there are additional mechanisms underlying mussel effects on sediment transport that I did not measure.

The National Research Council recently addressed the need to develop a better mechanistic understanding of how biota influence physical transport processes (NRC 2010), and my results suggest hypotheses for future study. First, organisms that are active burrowers are likely to disrupt cohesive forces of the streambed itself, decreasing the critical shear stress required to initiate sediment entrainment. Second, organisms that produce exposed physical structures, such as shells or nets, should increase near-bed turbulence that could promote erosion. In my experiment, the *Actinonaias ligamentina* treatment was the monoculture with most gravel erosion at both densities (Fig. 4), and this species was also the most active burrower in the experiment (Allen & Vaughn 2009). Furthermore, as a smooth shelled species, *A. ligamentina* lacks any shell sculpture that could reduce turbulence generated by its exposed shell (Watters 1994). Third, the structural complexity of the physical structures produced by biota, such as the presence of anchoring or anti-scouring sculptures on mussel shells, could further modify the effect of organisms on near-bed hydraulics. In this study, *Amblema plicata* and *Quadrula pustulosa* monocultures had the lowest amount of gravel erosion. Neither species are active burrowers, and both have anti-scouring and anchoring shell morphologies (Watters 1994). Fourth, these traits have the potential to interact in non-additive ways when multiple species are present. In this study, two high-density polycultures had significant non-additive

increases in erosion when compared to their respective monocultures (Fig. 4). Because these mussel species differ in size, shell shape (smooth vs. ridges vs. pustules), and burrowing depth, these traits may interact to generate a more topographically complex surface that can increase turbulence similar to what has been observed with caddisflies (Cardinale et al. 2002). Additional studies are necessary to test these hypotheses.

Finally, it is important to consider the design and scale of our experiment when interpreting and extrapolating our results. While the hydraulic conditions in our artificial streams appear to scale to some aspects of at least one natural stream (Table 1), there are other aspects of our flumes that are by necessity unrealistic: our flumes are much smaller than a natural mussel bed that can be several thousand square meters in area, our experiment was conducted at a relatively short time frame, and we only manipulated flows at two different velocities. Because of these limitations, we are unsure if we would observe the same results at the larger scale of a natural river with a wider range of flows. In addition, because mussel habitat in rivers is patchily distributed and limited to areas of low scour during high flow events, mussel effects on sediment transport are likely localized and are also likely to be small relative to sediment transport dynamics within an entire watershed. An in-depth field study is necessary to understand how mussels influence erosion at larger spatial and temporal scales.

BEF studies often focus on the effects of species richness because of the worldwide extinction crisis, but biodiversity losses also include declines in abundance of common species, and shifts in species dominance patterns (Hooper et al. 2005). Because common species are typically drivers of ecosystem processes (Moore 2006),

such declines have profound implications for ecosystem function. My results show that declines in abundance can also modify how biodiversity affects ecosystem processes. Further, thjis study shows that the structure of biological communities can influence physical transport processes, which is central to improving our understanding of how ecosystems and landscape processes are linked.

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TABLES

Table 1. Comparison of the estimated hydraulic variables describing flow conditions in our flumes versus the range of those measured in mussel beds in the Little River, Oklahoma. Data from the Little River, OK, is from Allen and Vaughn (2010). For our flumes, low and high flows refer to flow velocities of 0.05 m/s and 0.83 m/s. For data from the Little River, low and high flows refer to mean discharges of 0.63 m^3/s and 53.07 m^3/s (which correspond to percent exeedances of 95.2 and 27.0, respectively).

	Low Flow		**High Flow**	
Variable	*Flume*	*Little River, OK*	*Flume*	*Little River, OK*
Froude number	0.03	0.00 – 0.43	0.46	0.03 – 0.53
Reynolds number	1.65×10^4	$0 – 10.80 \times 10^4$	2.74×10^5	$1.53 \times 10^5 – 35.28 \times 10^5$
Boundary Reynolds number	81.8	0 – 7323	1360	24.46 – 14730
Shear Stress ($dynes/cm^2$)	0.15	0.078 – 131.22	40.4	0.88 – 628

Table 2. Summarized results from contrasts testing for biodiversity effects in polycultures. "Liberal" contrasts test the null hypothesis that polyculture performance can be predicted additively from performance of its monocultures. "Conservative" contrasts test the null hypothesis that polyculture performance is the same as its monoculture with the strongest effect on gravel erosion (see methods). Partial omega squared (ω^2) estimates effect size (see methods), bold values highlight $P < 0.05$, "High" and "Low" refer to density treatments, "Act" refers to *Actinonaias ligamentina*, "Amb" refers to *Amblema plicata*, "Quad*"* refers to *Quadrula pustulosa*, and "3 spp." refers to the 3-species polyculture.

Liberal				*Conservative*			
Contrast	$\boldsymbol{F}_{1, 132}$	$\boldsymbol{\omega^2}$	***P***	**Contrast**	$\boldsymbol{F}_{1, 132}$	$\boldsymbol{\omega^2}$	***P***
Act + Amb, Low	0.532	—	0.463	Act + Amb, Low	0.177	—	0.675
Act + Quad, Low	0.001	—	0.973	Act + Quad, Low	0.067	—	0.796
Amb + Quad, Low	0.169	—	0.682	Amb + Quad, Low	0.117	—	0.732
3 spp., Low	2.555	0.061	0.112	3 spp., Low	1.017	0.001	0.315
Act + Amb, High	0.002	—	0.962	Act + Amb, High	1.857	0.033	0.175
Act + Quad, High	0.112	—	0.739	Act + Quad, High	3.146	0.082	0.078
Amb + Quad, High	6.393	0.183	**0.012**	Amb + Quad, High	4.451	0.126	**0.037**
3 spp., High	5.150	0.147	**0.025**	3 spp., High	0.005	—	0.944

Table 3. Summary of stepwise multiple regression analysis of net change in substrate erosion (g) using a suite of burrowing variables (see methods), only the 2 significant models are shown.

Model/Variable	**Adjusted-R^2 or ß**	***F*/*t***	***P***
Model 1	$R^2 = 0.113$	$F_{1, 166} = 22.341$	< 0.001
- Mussel orientation relative to flow direction	ß = 0.344	$t = 4.727$	< 0.001
Model 2	$R^2 = 0.129$	$F_{2, 165} = 13.336$	< 0.001
- Mussel orientation relative to flow direction	ß = 0.323	$t = 4.414$	< 0.001
- S.D. surface area added	ß = 0.145	$t = 1.984$	0.049

FIGURES

Figure Legends

Figure 1. Diagram of the flumes used in chapter 2.

Figure 2. Mussel species (Bivalvia: Unionoida: Unionidae) used in chapter 2: A) *Actinonaias ligamentina*, B) *Amblema plicata*, C) *Quadrula pustulosa*. Note that the scale bar differs in size for each species. Photos courtesy of the Illinois State Museum.

Figure 3: Log-transformed gravel erosion (g) as a function of species richness for low-density (A) and high-density (B) treatments. Regression lines are: A) $y = 1.704 + 0.088x$, $P = 0.002$, $R^2 = 0.08$, B) $y = 1.66 + 0.070x$, $P = 0.045$, $R^2 = 0.04$. Note the different scales on y-axis for panels A and B, and that data points are jittered about the x-axis.

Figure 4: Boxplots of the net change in gravel erosion (g) relative to controls for mussel diversity treatments. White and gray boxplots designate low and high-densities, respectively, and the solid horizontal line represents the control mean value. "Act" refers to *Actinonaias ligamentina*, "Amb" refers to *Amblema plicata*, "Quad*"* refers to *Quadrula pustulosa*, and "3 spp." refers to the 3-species polyculture. Asterisks above a boxplot (*) designate a significant liberal non-additive biodiversity contrast for that treatment, and the pound sign (#) denotes a significant conservative non-additive biodiversity contrast for that treatment.

Figure 1.

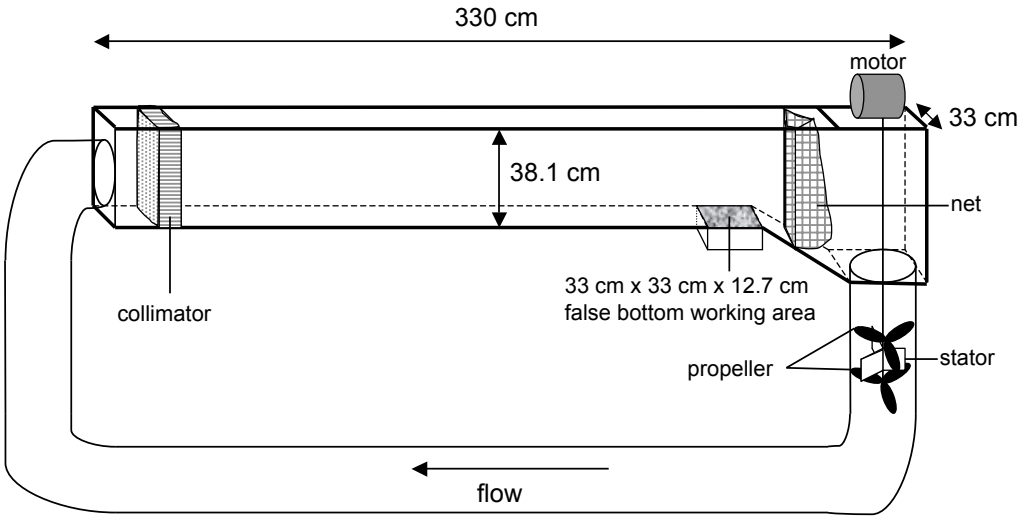

330 cm
motor
33 cm
38.1 cm
net
collimator
33 cm x 33 cm x 12.7 cm
false bottom working area
propeller
stator
flow

Figure 5.

Figure 3.

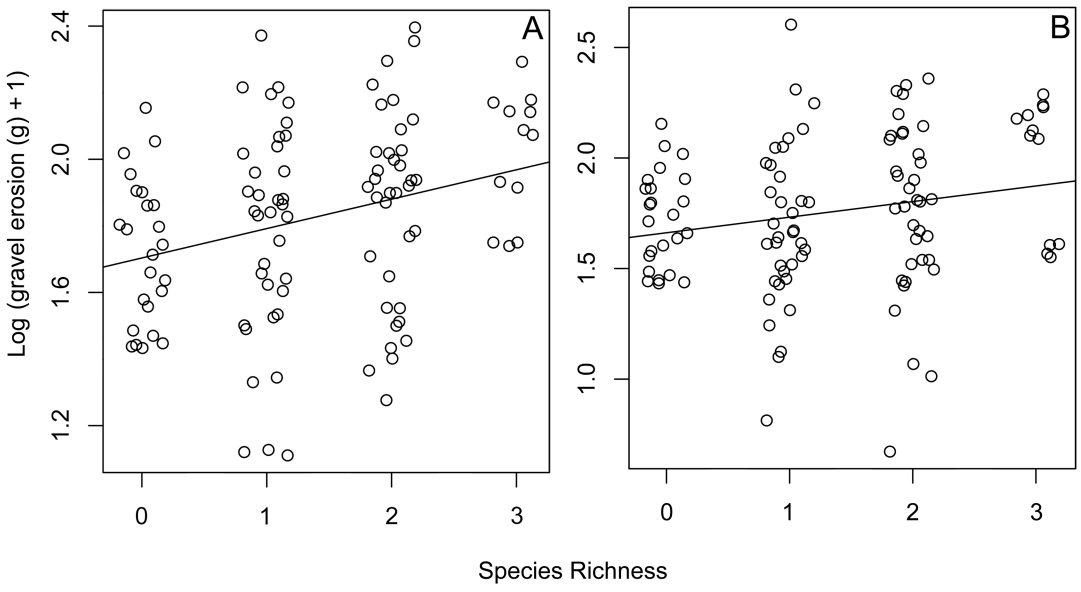
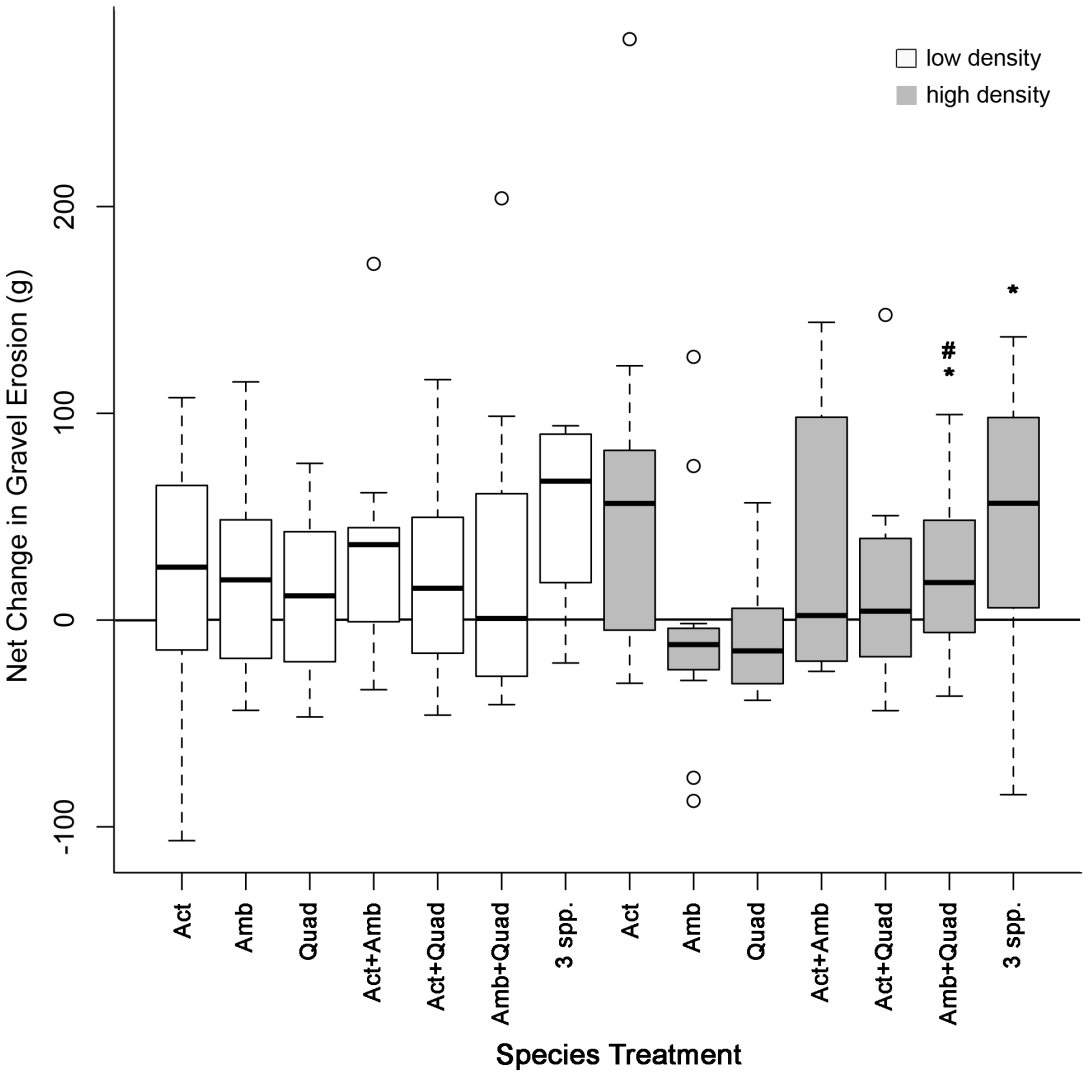

A
B
Log (gravel erosion (g) + 1)
1.2
1.6
2.0
2.4
1.0
1.5
2.0
2.5
0
1
2
3
Species Richness

Figure 4.


low density
high density
Net Change in Gravel Erosion (g)
-100
0
100
200
Act
Amb
Quad
Act+Amb
Act+Quad
Amb+Quad
3 spp.
Act
Amb
Quad
Act+Amb
Act+Quad
Amb+Quad
3 spp.
Species Treatment

CHAPTER 3

Biodiversity driven productivity cascades across ecosystem boundaries

Abstract: Biodiversity can increase ecosystem productivity and adjacent ecosystems are often linked by the flow of energy and nutrients between them. However, little is known about how biodiversity affects the flow of resources between ecosystems. Here I describe the influence of freshwater mussel biodiversity on a complex trophic cascade from streams to riparian forests. Mussel biodiversity increases algae production in streams, which is followed by increases in abundance of grazing aquatic insect larvae. Because adult aquatic insects are an important prey subsidy to terrestrial predators, mussel biodiversity may be increasing the flow of resource subsidies from aquatic to terrestrial habitats. In a mesocosm experiment I found that mussel species richness was associated with an increase in algae production rates, aquatic insect emergence rates, and spider standing crop biomass. Effects of mussel polycultures on algae production could be predicted additively from monocultures, and mussel effects were linked through stable isotope analyses to mussel-derived nitrogen subsidies. In contrast, certain mussel species mixtures had non-additive effects on insect emergence. Mussel polycultures were associated with a more evenly distributed algae community than mussel monocultures, and aquatic insect emergence rates were higher in these more mixed algal assemblages. Finally, spider standing crop biomass weakly tracked increases in aquatic insect emergence. In a field study of mussel communities on 2 rivers we found that sites with greater mussel species richness had higher aquatic insect emergence rates. These results show that because food webs in adjacent ecosystems are often linked, effects of biodiversity losses are not limited to ecosystems in which extinctions occur.

INTRODUCTION

Several decades of studies investigating the relationship between biodiversity and ecosystem function (BEF) have consistently shown that biodiversity can increase ecosystem productivity (Balvanera et al. 2006, Cardinale et al. 2006). To date, most BEF experiments have been conducted within a single, lower trophic level, but BEF studies need to include multiple trophic levels to better understand the role of biodiversity in complex ecosystems (Duffy et al. 2007). Although some BEF studies have begun to incorporate trophic complexity (Douglass et al. 2008, Griffin et al. 2008), these studies have been conducted within a single ecosystem or habitat type. Thus, BEF studies have yet to account for the general consensus that food webs in adjacent ecosystems are often linked (Polis et al. 1997), which suggests that the consequences of biodiversity losses on ecosystem functioning may not be limited to the ecosystem in which an extinction occurs.

Fusing elements of landscape and food web ecology, recent studies have shown that the flow of energy and nutrients between ecosystems is common (Marczak et al. 2007), and the term "resource subsidy" has been given to the movement of nutrients, detritus, or prey across ecosystem boundaries. Streams and riparian forests are a model system for examining resource subsidies because they are characterized by reciprocal flows of energy and nutrients (Baxter et al. 2004, Baxter et al. 2005, Marczak et al. 2007). Terrestrial inputs drive production in smaller streams, and their effects extend to top trophic levels (Vannote et al. 1980, Wallace et al. 1997). Resource subsidies also flow from aquatic to terrestrial habitats, as recent studies have documented the importance of emergent aquatic insect subsidies from streams to

riparian predators (Nakano and Murakami 2001, Sabo and Power 2002, Power et al. 2004). We know that top-down trophic cascades in streams via predator addition can reduce aquatic insect subsidies to riparian food webs (Baxter et al. 2004, Wesner 2010). However, investigations into whether bottom-up trophic cascades in streams can enhance aquatic insect subsidies to riparian food webs are lacking despite the results of recent studies showing that bottom-up forces in aquatic ecosystems can be strong enough to mitigate top-down effects (Chase 2003, Wojdak 2005, Blanchet et al. 2008). Further, the majority of our understanding of how community structure influences resource subsidy flux between ecosystems comes from studies that add or remove representative species of an entire trophic level (Knight et al. 2005, Wesner 2010), neglecting the now commonplace understanding that non-additive species interactions can drastically modify the flow of energy and nutrients in food webs (Balvanera et al. 2006, Cardinale et al. 2006).

Here I examine how species diversity of an important group of consumers can impact the flow of resource subsidies across aquatic and terrestrial ecosystems. Freshwater mussels (Bivalvia: Unionidae, hereafter "mussels") are a diverse guild of long-lived (6-100 y), burrowing, filter-feeding bivalves that live in river sediments. Mussels occur in dense, speciose aggregations ("mussel beds") that are patchily distributed in streams, and can dominate benthic biomass by an order of magnitude (Strayer 2008). Mussel communities increase standing crops of benthic algae by excreting nutrients that fertilize algae (Vaughn et al. 2007, Spooner and Vaughn 2008). Further, mussel communities are associated with increased abundances of grazing aquatic insect larvae, which are likely tracking the increases in algae caused

by mussels (Spooner and Vaughn 2006, Vaughn and Spooner 2006, Vaughn et al. 2008). Because mussel biodiversity modifies mussel effects on primary producers (Vaughn et al. 2007), and because grazing aquatic insects link aquatic and terrestrial food webs (Baxter et al. 2005), mussel communities are a good system for testing hypotheses linking the concepts of biodiversity and ecosystem function with the flow of resource subsidies across ecosystem boundaries.

I performed experiments examining the effects of mussel species richness on a trophic cascade that influences the flux of aquatic insect prey subsidies to riparian predators. I hypothesized that: 1) mussel species richness will increase primary production because mussel species vary in the quantity of mussel-derived nutrients subsidized to benthic algae, 2) grazing aquatic insect larvae will respond to increases in primary production, so the emergence rates of adult aquatic insects into terrestrial ecosystems will also increase with mussel species richness, and 3) terrestrial predators that specialize on aquatic insect prey subsidies, such as riparian spiders, will respond to increases in aquatic insect emergence rates and will also increase with mussel species richness.

METHODS

I tested my hypotheses with a mesocosm experiment and comparative field study. The mesocosm experiment allowed me to directly quantify mussel biodiversity effects and also investigate the mechanisms underlying these effects by using stable isotopes to track mussel-derived nutrients through food webs. The comparative field study allowed me to verify that the patterns I observed in the mesocosms also occurred in a more complex natural system.

Mesocosm experiment:

Design and sampling: I conducted an 8-week experiment in July – August 2010 in 40 recirculating mesocosms housed in a greenhouse. Mesocosms consisted of molded plastic liners (0.6 m2) suspended in a fiberglass basin to allow water circulation below and around the liner, simulating natural stream flow (Allen and Vaughn 2009). Flow (~13.7 cm/s) was maintained with 1/32 horsepower pumps. Mesocosms contained an ~8.9 cm layer of gravel (10 – 25 mm diameter) and 26.7 cm pond water, for an approximate volume (gravel plus water) of 170 L. I filled mesocosms 3 weeks prior to the experiment to allow colonization by aquatic insects and algae, and we conducted biweekly 50% partial water changes. Throughout the experiment I added a cultured algal mixture (dominated by Scenedesmus spp., mean chlorophyll a = 0.212 mg/L) daily to the mesocosms; 200 mL per mesocosm in weeks 1-3 until algae visibly established in the benthos and 100mL per mesocosm in weeks 4-8 to sustain mussels.

I collected 3 mussel species (*Actinonaias ligamentina* [n = 102], *Amblema plicata* [n = 96], and *Quadrula pustulosa* [n = 106]) from a single site on the Kiamichi River, Pushmataha Co., Oklahoma, U.S., and all mussels were measured for length and individually tagged. We chose these species because they are regionally common, co-occur in mussel communities, but differ in physiological traits that influence nutrient excretion. *Actinonaias ligamentina* and *Q. pustulosa* are thermally sensitive species that are catabolic at summer temperatures (~ 30° C, *A. ligamentina* is considerably more catabolic than *Q. pustulosa*), while *A. plicata* is thermally tolerant and anabolic at summer temperatures (Spooner and Vaughn 2008). These differences

in physiology result in different nitrogen excretion rates, and likely different fertilization effects on primary producers (Spooner and Vaughn 2011).

I used the 3 mussel species to generate 8 mussel species treatments: 1 no-mussel control, 3 monocultures (single-species treatments), and 4 polycultures (3 two-species and 1 three-species treatments). Mussels were randomly assigned to treatments to maintain constant mussel densities of 6 per mesocosm (corresponding to a density of ~10 mussels/m2, a naturally occurring, low density for mussel beds in the region). I used a substitutive design at a constant density to maximize evenness and incorporate as many monocultures as possible to test for non-additive biodiversity effects in polycultures (Schmid et al. 2002). The mussel species used vary in size (mean lengths ± SD [mm]: Actinonaias ligamentina, 103 ± 10.7; Amblema plicata, 84 ± 6.3; Quadrula pustulosa, 49 ± 5.4), which could also influence the quantity of mussel-derived nutrient subsidies in addition to species physiological traits. Accordingly, I estimated the mussel biomass of each treatment using length-dry weight regressions from (Vaughn et al. 2007) and used mussel biomass as a covariate in statistical models to account for biomass differences. During the experiment, stressed (valves would not close when touched) mussels were replaced (mesocosms were checked daily).

Mesocosms were arranged in 5 blocks of 8 mesocosms, with each block increasing in distance from an evaporative cooler at the north end of the greenhouse, and mussel treatments were assigned to mesocosms in a randomized block design. The cooler causes a slight temperature gradient within the greenhouse, and also serves as a source of aquatic insect colonists that live in cooler holding tanks. The mean midday water temperature (± S.D.) in mesocosms over the experiment was 29.7º ± 0.99ºC,

which is in the range of summer midday temperatures for Kiamichi River mussel beds (Vaughn et al. 2007), and the mesocosms in the block furthest from the cooler were 1º C warmer on average.

I used 2.5 cm diameter silica disks (3 per mesocosm) and 232.3 cm^2 unglazed clay tiles (1 per mesocosm) as colonization surfaces for benthic algae. Both substrates were sampled weekly and replaced. Silica disks were frozen and chlorophyll *a* was extracted using the acetone method and analyzed spectrophotometrically with a correction for pheophytin (ASTM 1995). Algae production rate was calculated as mg chl *a* per m^2 per day. Clay tiles were scrubbed in distilled water to create a slurry that was passed through a 88 μm sieve to remove macroinvertebrates and then filtered through a pre-combusted 0.45 μm pore glass-fiber filter. Because this slurry contained bacteria and other microorganisms in addition to periphyton, we refer to these samples as *phytomicrobenthos*, or "PMB." Filters were frozen and PMB was analyzed for N^{15} enrichment as described below.

Each mesocosm contained one floating trap to sample emergent aquatic insects (Fig. 1). Emergence traps consisted of a galvanized steel frame and 0.2 mm mesh with a collecting bottle at the top with an inverted funnel containing a small piece of insecticidal strip (active ingredient: dichlorvos; Hot Shot No-Pest Strip, United Industries, St. Louis, Missouri, USA) following Wesner (2010). Each trap had a collection area of 685 cm^2. We sampled emergence traps biweekly beginning in week 2. Insects were preserved in ~ 75% EtOH, identified to family following (Merritt et al. 2009), enumerated and their length measured for estimation of biomass using regressions in Sabo et al. (2002). Aquatic insect biomass was dominated by

chironomids (primarily algivorous tube-dwellers in the tribe Chironomini). We calculated insect emergence rates as mg per m^2 per day.

During week 3, I introduced 120 tetragnathid spiders to the greenhouse (3 on each mesocosm) that were collected from single site on Byrd's Mill Creek near Fittstown, OK. Beginning in week 4, I surveyed each mesocosm for spiders approximately 2 hours after sundown when spiders where most active (2 spider surveys per week, 10 in total). Because spiders reproduced during the experiment, during surveys I classified each spider as belonging to one of four size classes (spiderling, juvenile, small adult, large adult). At the end of the experiment, I collected all spiders and preserved them in ~75% EtOH. I later freeze-dried a subsample of spiders in each size class, weighed them to the nearest 0.01 mg, and used these data to estimate spider standing crop biomass for each mesocosm during each survey (large adults: n = 10, mean weight = 13.01 mg; small adults: n = 14, mean = 4.87 mg; juveniles: n = 23, mean = 1.99 mg; spiderlings: n = 25, mean = 0.67 mg).

To test if mussel effects on benthic foodwebs were due to the addition of mussel-derived nutrients, I used a stable isotope approach with N^{15}. Mussels were held in a Living Stream holding tank (in a separate greenhouse to prevent N^{15} contamination of the mesocosms) and fed a cultured algal mixture enriched in N^{15} (~ 100 δ N^{15} ‰ relative to air) for 13 weeks. Samples of hemolymph analyzed for N^{15} showed that mussels had mean δ N^{15} values of 6.06 ‰ prior to being fed the enriched diet. Two weeks prior to adding mussels to the mesocosms, their shells were cleaned of biofilm and they were moved to a holding tank in the experimental greenhouse. Mussels were starved to remove any enriched algae from their digestive tract, water

was changed daily, and shells were cleaned again before mussels were added to mesocosms.

Stable isotope analysis of mussel hemolymph taken at the end of the experiment showed that mussels maintained an enriched level of N^{15}, but mussel species differed in δN^{15} values (ANOVA, $F_{2, 12} = 4.47$, $P = 0.035$; mean δN^{15} ‰ values: *Actinonaias ligamentina* = 10.08, *Amblema plicata* = 9.62, *Quadrula pustulosa* = 13.08). Pond water used to fill mesocosms had a mean δN^{15} ‰ value of 9.51, while cultured algae added to mesocosms was depleted in N^{15} (mean 0.43 δN^{15} ‰), and together these sources represent the background N^{15} enrichment of mesocosms. To account for mussel species differences in N^{15} enrichment, I first wanted to standardize δN^{15} ‰ values of PMB and aquatic insect samples to the background levels represented by no-mussel controls that only received pond water and cultured algae. To do so, I took the difference in δN^{15} ‰ values of samples from mussel treatments from the mean δN^{15} ‰ value of the no-mussel controls (which I refer to as the "standardized δN^{15} ‰ value") We then estimated the δN^{15} ‰ value of the nitrogen pool available to be provided by mussels in each mussel treatment by using the mean δN^{15} ‰ value of mussel hemolymph for each species (which I refer to as the "δN^{15} ‰ value of the mussel pool"). If the mussel treatment was a polyculture, the mean δN^{15} ‰ value was weighted by the proportion of mussel biomass that species contributed to the species mixture. Finally, I divided the standardized δN^{15} ‰ value by the N^{15} ‰ value of the mussel pool, which gave me a metric describing the relative enrichment of the sample to the nitrogen pool available within the mussels themselves. I refer to this metric as the "Mussel Derived Nitrogen Index," as a higher value indicates the sample

has assimilated more mussel-derived nitrogen. I analyzed PMB and emergent aquatic insect samples collected in week 8 for N^{15} enrichment. For PMB, I freeze-dried glass-fiber filters containing PMB samples, and scraped a portion of the sample off the filter that was packed into a tin capsule. For emergent insects, I freeze dried the sample and then selected 6-10 chironomids that were packed into a tin capsule. Samples were analyzed for stable isotope ratios at the stable isotope laboratory at the University of Oklahoma.

At the beginning of week 3 I noticed some visual differences in benthic algae communities among mussel treatments (some appeared to be dominated by green filamentous algae, and others by brown diatoms). I took ad hoc samples of algae from each mesocosm for identification at the end of week 7. I sampled benthic algae by selecting two pieces of gravel from each mesocosm and scraping a small section of the east wall of every mesocosm; the gravel with attached algae and scraping were preserved in ~ 30% formalin. Later, each algae sample was homogenized and algae subsamples were identified to genus. I subjectively ranked each algae taxon on a 10-point log-scale of biomass following methods described in (Biggs and Kilroy 2000).

Statistical analysis of treatment effects: I analyzed the relationship between mussel species richness and response variables on log-transformed data from the three trophic levels (mean algae production rate from 8 weekly samples, mean insect emergence rate from 14 biweekly emergent insect samples in weeks 2-8, and mean spider standing crop biomass from 10 biweekly spider surveys in weeks 4-8) with linear regressions. To test if species treatments differed in their effects on algae production, aquatic insect emergence rates, and spider standing crop biomass, I ran

one-way ANCOVAs on the log-transformed means from the whole experiment as above. Although I could have increased our statistical power by using a repeated-measures analysis, I wanted to include up to 2 potentially important covariates (mussel biomass and spatial block). Other experiments have shown mussel biomass to be an important covariate in mussel effects on primary production (Vaughn et al. 2007), and I used a randomized block design to account for a known temperature gradient in the greenhouse and distance from a source of aquatic insect colonists. While mussel biomass showed a linear relationship to mean overall algae production rates, and block number showed a linear relationship to mean overall aquatic insect emergence rates, these relationships did not hold throughout all 8 algae samples or all 14 emergent insect samples. Because I was interested in overall effects throughout the experiment (not differences between points of time within the experiment), I sacrificed some statistical power to account for these covariates. I fitted general linear models with mussel species as a fixed effect with covariates individually, both in combination, and with no covariate; and used the model that best fit the data as determined by Akaike's Information Criterion (AIC).

Following other BEF experiments (Douglass et al. 2008, Allen and Vaughn 2011), I conducted 8 *a priori* planned linear contrasts to test for non-additive biodiversity effects if our general linear model was significant. The first set of contrasts (n = 4) tested the null hypothesis that the observed polyculture mean is the same as the expected mean based on additive monoculture performances (i.e. a two species polyculture treatment was given a contrast coefficient of 1, while the 2 monoculture treatments of the species present in that polyculture were given contrast

coefficients of –0.5; testing the null hypothesis that the mean of the polyculture was equal to the weighted means of its monocultures). The second set of contrasts (n = 4) compared the observed polyculture performance against its monoculture with the strongest effect. These two types of contrasts represent a liberal and a conservative test for non-additive biodiversity effects, respectively, and I refer to them as such.

Table-wide adjustments have been recommended to decrease the increased probability of Type I errors when performing multiple comparison procedures (Rice 1989), but recently others have criticized such methods for obscuring ecologically significant effects and increasing Type II errors (Moran 2003, Nakagawa 2004). Therefore, I followed (Douglass et al. 2008) and opted not to apply a table wide adjustment of *P*-values to our contrasts, but rather I report the effect size of each test along with exact *P*-values whenever possible, using both to interpret ecological significance (i.e. if the results of a contrast was statistically significant but had a small effect size, I would view the result with caution). Further, I interpret the results of multiple statistical tests strictly within the context of our single broader hypothesis (i.e. whether or not non-additive biodiversity effects are present). Effect sizes are reported as Cohen's d, which measures the difference between group means relative to the standard deviation, with values near 0.2 and 0.3 considered "small" effect sizes and those greater than 0.8 considered "large" (Cohen 1988).

I collected 23 genera of benthic algae. Because I was interested in mussel influences on shifts in algae taxa dominance patterns (rather than presence/absence of rare taxa), I reduced our data to the 10 most common algae taxa (which were present in at least 80% of all samples). We then used non-metric multi-dimensional scaling

(NMDS) with the Sorensen distance measure (Bray-Curtis) and multiple response permutation procedure (MRPP) in PC-ORD to compare algal communities between mussel species treatments. The MRPP determines the treatment effect size (A) where $A = 1$ indicates similarity and $A = 0$ indicates heterogeneity among treatments. The NMDS Axis 1 had the highest R^2 (0.62) of the final stable two-axis solution, and also clearly separated algae communities along a gradient of diatom relative abundance. Therefore, I used this axis in further analyses to investigate how algae community dominance shifts towards diatoms were related to mussel-derived nutrients and aquatic insect emergence rates.

Statistical analysis for mechanisms underlying mussel effects: To test if mussel-derived nutrients are a likely mechanism for mussel effects on algae and aquatic insects, I ran linear regressions on log-transformed algae production and aquatic insect emergence rates during week 8, using the Mussel Derived Nitrogen Index (as described above) from PMB or aquatic insect samples from those weeks as the predictive variable. To determine if mussel-derived nutrients were influencing algae community species composition, I ran linear regressions on NMDS axis 1 using PMB Mussel Derived Nitrogen Index as the predicting variable. To determine if aquatic insect emergence rates were responding to changes in algae production rates or changes in algae community composition, or both, I fit linear and quadratic regression models using algae production rates and NMDS axis 1 to predict aquatic insect emergence rates using treatment block as a covariate. To see which model best-explained aquatic insect emergence rates (n = 8 models with all possible single and paired combinations of algae production rates and NMDS axis 1 as either a linear or

quadratic relationship), I used a model selection approach using AIC. We report Δ_i as the AIC difference between a given model and the best performing model and Akaike weights, w_i, which describing the relative likelihood that a particular model is the best model out of all models (Burnham and Anderson 2002). Finally, I also ran a linear regression to see if standing crop spider biomass was responding to aquatic insect emergence rates.

Comparative Field Study

Design and sampling: I established 9 study sites on 2 rivers in SE Oklahoma (5 on the Little River, 4 on the Kiamichi River) that span a gradient of mussel species richness (Galbraith et al. 2008, Allen and Vaughn 2010). At each site I established a 100-m study reach, and deployed eight 0.33 m^2 emergent aquatic insect sampling traps constructed out of PVC pipe, foam floats, and 0.2 mm mesh following (Malison et al. 2010). Emergence traps were loosely secured to a rebar stake with a zip-tie to allow the trap to rise and fall with changes in water level. Emergence traps were haphazardly placed throughout the site, restricted to locations near mussels and not more than 50 cm in depth in order to accommodate a 45 cm rise in water level. Each trap had a mesh catch near the top to capture emerging insects that would have otherwise been lost to rain, wind, or mortality. Insects were removed from traps with an aspirator and preserved with ~75% EtOH. Traps were deployed on July 16 and July 17, 2009, and sampled one week later. The study period was restricted to a single week because of flooding that washed away emergence traps. Insects were enumerated, identified to order or family, measured for length, and biomass estimated using length-mass

regressions from (Sabo et al. 2002). Emergence rates were calculated as insect mg per m^2 per day.

I sampled mussels at each site by excavating one 0.25 m^2 quadrat at each emergence trap (n = 8), and conducting a 120-minute timed search spanning the 100-m reach at each site. Mussels were brought to shore, identified, and returned alive to the streambed. I used these data, which were corroborated from data from other recent surveys at these sites (Galbraith et al. 2008, Allen and Vaughn 2010), to estimate mussel species richness.

I measured physical habitat variables at each site to account for habitat differences among sites. At each site I measured the water line slope with a surveyor's level, and at each trap I measured water depth (d), flow velocity (U, measured at 0.6 d), qualitatively described substrate composition (% boulder, % cobble, % sand, and % silt), and estimated substrate roughness using a 30.5 cm chain (3-mm links). Following Hardison and Layzer (2001), the substrate roughness (k) was calculated as $k = 3.5/c$, where c is the linear distance between the two ends of the chain (cm) after following the contours of the substrate. Using measurements of water depth, flow velocity, and substrate roughness, I estimated the hydraulic variables Reynolds number (Re), boundary Reynolds number (Re_*), Froude number (Fr), and shear velocity (U_*) using formulae in Statzner et al. (1988, Table 1).

Statistical Analysis: I collected five orders of adult aquatic insects in our emergence traps: Diptera, Trichoptera, Ephemeroptera, Plecoptera, and Odonata. I removed Odonates from our analysis since they are predators and we were interested in potential bottom-up effects of mussels on primary consumers. Additionally, I

omitted Ephemeropterans and Plecopterans because they comprised a very small portion of overall biomass (1.6 and 1.7%, respectively) and were not present in all samples (60 and 48%, respectively).

Little River sites had more mussel species than Kiamichi River sites (mean of 15.4 mussel species versus 12.5). Further, aquatic insect emergence rates were higher on the Little River than on the Kiamichi River (mean emergence rate of 83.51 $mg \cdot m^{-2} \cdot d^{-1}$ versus 36.19 $mg \cdot m^{-2} \cdot d^{-1}$). This could be due to differences in physical habitat in the two rivers, as the Little River is larger with higher discharge and a less rocky streambed. I wanted to test the hypothesis that insect emergence rates were higher at sites with greater mussel species richness, irrespective of habitat differences between the 2 rivers. Therefore, I standardized emergence rates to account for differences in physical habitat among the rivers, and among sites within each river. I used a model-building information theoretic approach, developing a suite of multiple regression models using physical habitat variables measured at each trap (water depth, water velocity, slope of water line, % bedrock, % boulder, % cobble, % gravel, % sand, % silt, *Re*, *Fr*, U_*, and Re_*) on log (x + 1) emergence rates of each taxon (Diptera and Trichoptera) separately for each river. If physical habitat variables were multicollinear ($r > 0.85$), I kept the variable that was most correlated with the taxon of interest. I used AIC to determine the best set of candidate models, and used an averaged model (using all models with $\Delta_i < 2$) to predict emergence rates based on physical habitat variables alone. I summed the residuals from taxon-specific averaged models to generate a single residual for aquatic insect emergence rates at each trap on each river (Table 2). I refer to the residuals as "standardized aquatic insect emergence rates" because they

standardize for differences in physical habitat among rivers, and among sites within rivers. Further, a given value can be interpreted as having either a higher or lower emergence rate based on physical habitat alone if it is positive or negative, respectively.

I wanted to test for a positive relationship between median standardized emergence rate and mussel species richness, but I also wanted to account for unmeasured differences between rivers (that I did not measure while standardizing emergence rates). Therefore, I fit a mixed effect linear regression model with the general formula of $y_{ij} = \alpha + \beta x_{ij} + a_j + \varepsilon_{ij}$, where y is the median standardized emergence rate at each site, x is the mussel species richness at each site, α and β are the fixed slope and intercept, a is the random effect of river on the intercept, i is sampling site and j is river. This mixed-effects linear model assumes the same β for mussel species richness on each river, but incorporates a random effect to allow for random variation of the intercept (α) at each river.

Results:

Mesocosm experiment: *Treatment effects:* Linear regressions showed significant increasing relationships between mussel species richness and algae production rates ($y = 0.729 + 0.036x$, $P = 0.029$, $R^2 = 0.120$), aquatic insect emergence rates ($y = 1.930 + 0.160x$, $P < 0.001$, $R^2 = 0.277$), and spider standing crop biomass ($y = 1.217 + 0.039x$, $P = 0.010$, $R^2 = 0.161$). The magnitude of the species richness effect varied among trophic levels, as the increase in the response variable from the no-mussel controls to 3-species polycultures was 46% for algae production, 89% for aquatic insect emergence rate, and 26% for standing crop spider biomass (Fig. 2). The

ANOVA models showed that mussel species treatments significantly differed in algae production rates ($F_{7, 31} = 5.446$, $P < 0.001$; mussel biomass covariate: $F_{1, 31} = 3.166$, $P = 0.085$) and aquatic insect emergence rates ($F_{7, 31} = 5.000$, $P < 0.001$; block covariate: $F_{1, 31} = 14.185$, $P < 0.001$), but did not significantly differ in spider standing crop biomass ($F_{7, 32} = 1.497$, $P = 0.204$).

All planned contrasts testing for non-additive mussel biodiversity effects on algae production rates were insignificant (Table 3). For aquatic insect emergence rates, 2 of 4 liberal contrasts testing for non-additive biodiversity effects were significant, and 1 of 4 conservative contrasts was significant (Table 3). Because we did not find significant effects of mussel treatments on spider standing crop biomass, we did not test for mussel non-additive biodiversity effects on spiders. The magnitude of the non-additive mussel biodiversity effects on aquatic insect emergence rates was large. The *Actinonaias ligamentina* and *Amblema plicata* polyculture had a 117% higher aquatic insect emergence rate than expected given additive performances of its monocultures, while the *Quadrula pustulosa* and *Amblema plicata* polyculture had a 96% greater aquatic insect emergence rate than additive expectations.

Overall, algae community structure differed significantly between mussel treatments (MRPP: $A = 0.157$, $P < 0.001$, Fig. 3A). NMDS axis 1 explained the most variation in algae communities ($R^2 = 0.62$), and broadly separated samples by association strength with diatoms, with diatoms (*Gomphonema, Nitszchia,* and *Synedra*) positively related to axis 1 and green algae (*Cosmarium, Oedogonium, Pediastrum, Scenedesmus, Spirogyra* and *Tetraedon)* and the synurid *Mallomonas*

negatively related to axis 1. NMDS Axis 2 (R^2 = 0.18) separated samples into finer scales within the broader diatom/non-diatom groups.

Underlying mechanisms: Linear regressions showed significant positive relationships between algae production rates and the Mussel Derived Nitrogen Index of PMB from samples (y = 0.157 + 0.924x, p < 0.001, R^2 = 0.49, Fig. 4A). Further, the Mussel Derived Nitrogen Index of PMB was positively related to NMDS axis 1 (y = – 0.796 + 3.288x, p = 0.004, R^2 = 0.22, Fig. 4B). We did not find significant relationships between emergence rates and δ N^{15} ratio of emerged insects (y =1.776 – 1.337x, P = 0.278, R^2 = 0.04). However, a quadratic model describing a unimodal concave-down relationship between NMDS Axis 1 and emergence rates was the best performing model (Akaike weight, w_i, = 0.99, and 0.72 after excluding a possible influential outlier), while models using algae production rates to predict aquatic insect emergence rates performed poorly (the highest w_i for a model including algae production rate was 0.008, Table 4). The quadratic model with NMDS axis 1 could explain 57% percent of the variation in aquatic insect emergence rates after including a block covariate (Fig. 3B). Finally, spider standing crop biomass showed a weak but significant positive relationship with aquatic insect emergence rates (y = 1.011 + 0.121x, P = 0.004, R^2 = 0.20; Fig. 4C).

Comparative Field Study

Models using physical habitat variables to predict emergence rates explained 3-70% of the variation in aquatic insect emergence rates (Table 2). The mixed effect linear regression showed a significant relationship between mussel species richness and standardized emergence rates at a site (y = – 1.70 + 0.118x, P = 0.006, R^2 = 0.69,

Fig. 5). Thus, after accounting for habitat differences, emergence rates were more than 5 times greater at the site with highest mussel species richness relative to the least speciose site, and mussel species richness at a site could explain nearly 70% of variation in emergence rates at that site.

DISCUSSION

Mussel species richness was associated with increases in primary producers and primary and secondary consumers. However, the nature of this relationship differed with trophic level. Effects of mussels on primary production appear to be driven by the strong effects of a single species. All planned contrasts testing for non-additive biodiversity effects on algae production rates were insignificant. This suggests that effects of mussel polycultures on algae production rates could be routinely predicted from the performance of monocultures. Because the mussel treatment with the highest algae production rates was the *Actinonaias ligamentina* monoculture (Fig. 2A), this indicates that increased algae production with higher mussel richness was driven by the repeated inclusion of this one high-performing species. In contrast, effects of mussels on primary consumers appear to be due to the synergistic effects of multiple species. Certain polycultures had significant non-additive effects with large effect sizes on aquatic insect emergence rates. My results suggest that different mechanisms underlie mussel effects on benthic primary producers and grazing aquatic insect consumers.

Benthic primary production was higher when algae were more enriched in N^{15}. Because the only source of enriched N^{15} was from mussels, this indicates that mussel-derived nitrogen was fertilizing algae and leading to increased algal production. Not

surprisingly, algae in mesocosms with *Actinonaias ligamentina* were more enriched in N^{15} than in mesocosms without (Fig. 4A). At warm temperatures, this thermally sensitive species catabolizes its own tissue and excretes ammonia at high rates (Spooner and Vaughn 2008), so I expected it to be the strongest contributor of mussel-derived nitrogen. In addition, other studies have found that *A. ligamentina* has particularly strong effects on benthic algae (Vaughn et al. 2007, Spooner and Vaughn 2011). I did not find a relationship between N^{15} enrichment and aquatic insect emergence rates, which may be an indication that the spike was not strong enough to transfer into the higher trophic levels. Alternatively, the mechanism driving mussel biodiversity effects on aquatic insects might be altogether different.

In addition to influencing primary production, mussel biodiversity also affected algal community composition. For example, the algal community in the *A. ligamentina* monoculture had the most diatoms while the *A. plicata* monoculture had the most filamentous green algae (Fig 10A). Stable isotope data suggest that changes in algal community composition were related to mussel-derived nitrogen (Fig 11B). It is likely that mesocosms with more mussel-derived nitrogen subsidies also had higher N:P ratios. Although I didn't measure water column nutrients in this experiment, other studies by have demonstrated higher N:P ratios with higher mussel ammonia excretion (Vaughn et al. 2004, Spooner and Vaughn 2008, Vaughn et al. 2008), and N:P ratios are known to be an important factor in determining competition outcomes in algae communities (Rhee and Gotham 1980).

My results suggest that mussel biodiversity changes algal community composition in ways that facilitate insect consumers. Mussel polycultures produced

more evenly distributed algal communities (Fig 10A) and consumer emergence rates were greatest from these mixed algae communities. The model that best explained insect emergence rates was a quadratic model using NMDS axis (a proxy for diatom relative abundance relative) that explained 57% of variation in emergence rates with the block covariate (Fig 10B). In contrast, the relatively poor performance of models using algal production as explanatory variables suggests that consumers were not responding to algae quantity. The dominant consumers in the mesocosms were algivorous chironomids which create tube-shaped retreats from silk and small particles (sand, organic matter, detritus, algae, etc.). Diatoms are more nutritious for grazers than filamentous green algae (Dodds 1991) and are more easily assimilated by chironomids (Berg 1995); chironomids were likely increasing in abundance as diatoms became more abundant. However, because I observed a unimodal relationship between insect emergence and NMDS axis 1, at some point an overabundance of diatoms was linked with a decline in emergence rates. This may reflect a preference for green filaments as tube-building material; Power et al. (2008) found that chironomids used green filamentous algae for physical habitat but fed on diatoms. The non-additive effects of mussel biodiversity on consumer emergence are probably due to the requirements of consumers for both high quality food and tube-building materials.

Finally, although I observed a significant relationship between mussel species richness and spider standing crop biomass, I did not find significant differences between mussel treatments themselves. Spider standing crop biomass only weakly tracked emergence rates (Fig 11C), which likely indicates that other factors (such as

available web-building habitat, spider movement patterns, or predation by other organisms residing in the greenhouse such as toads and jumping spiders) were more important in determining spider distributions on mesocosms.

Mussel species richness and aquatic insect emergence rates were also strongly associated in the comparative field study. Physical habitat variables like substrate type and flow velocity are strong regulators of aquatic insect distributions (Allan and Castillo 2007). We did not expect bottom-up effects of mussels on algae abundance and composition to override these physical influences, and the performance of our models using physical habitat variables varied with taxonomic group and river. However, after accounting for these habitat differences, mussel species richness explained nearly 70% of the remaining variation in insect emergence rates. These results are supported by a large-scale study of 30 sites in 8 rivers that found that mussel community structure explained 50% of the variation in benthic macroinvertebrate communities (Vaughn and Spooner 2006). More importantly, these results verify the robustness of the mesocosm experiment results.

My results should be interpreted within several limitations of this study. First, my data tracking the movement of N^{15} through the food web are derived from a mesocosm experiment that is by necessity simpler than the natural system. I don't have data confirming that these processes are also happening in the field, although mussel species that excrete nitrogen at higher rates stimulate higher benthic algal production in the field (Vaughn et al. 2007). Second, although my data suggest that consumers were responding to algal community composition, I did not verify this with gut content analyses or experiments examining preference for tube building materials.

Third, my field study was limited to a single week due to a high flow event. Although the results of the field study were consistent with those in the mesocosm experiment, I would have liked to examine these patterns over an entire summer. I did not sample algae in the field study and further work is needed to determine if mussel biodiversity influences algal composition in the field in ways that influence consumer production. Finally, because I did not manipulate species richness in the field study, I do not know if factors determining mussel species richness and aquatic insect emergence rates are the same (such as the productivity of a site). I believe a manipulate field experiment is an important next step to assuage some of these limitations.

Conclusion: BEF studies have consistently shown that non-additive species interactions are responsible for the increased productivity of ecosystems with greater species richness (Balvanera et al. 2006, Cardinale et al. 2006). Other studies have shown that because food webs in adjacent ecosystems are linked by flows of energy and nutrients, the productivity of one ecosystem can affect those next to it (Polis et al. 1997, Marczak et al. 2007). Here, I merge these two complementary research areas to show that biodiversity-driven productivity in one ecosystem can cascade across ecosystem boundaries to influence food webs in other ecosystems. This shows that because ecosystems are connected, the consequences biodiversity losses on ecosystem functioning are not limited to the ecosystem in which extinctions occur.

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TABLES

Table 1. Summary of hydraulic variables estimated at comparative field study sites. d is water depth, U is the mean water velocity (measured at 0.6 d), and k is substrate roughness.

Variable (symbol, unit)	**Formula**	**Description**	**Source**
Froude number (Fr, dimensionless)	$\sqrt{\frac{U^2}{gd}}$	ratio of inertial to gravitational forces	(Statzner et al. 1988)
Reynolds number (Re, dimensionless)	$\frac{Ud}{v}$	ratio of inertial to viscous forces	(Statzner et al. 1988)
Boundary Reynolds number (Re_*, dimensionless)	$\frac{U_* k}{v}$	roughness of flow near substrate	(Statzner et al. 1988)
Shear velocity (U_*, cm/s)	$\frac{U}{5.75 \log_{10}\left(\frac{12d}{k}\right)}$	friction velocity	(Statzner et al. 1988)

Table 2. Summary of the models using physical habitat variables to predict the emergence rates of aquatic insects at each emergence trap (only models with $\Delta_i < 2$ are shown). Δ_i is the AIC difference between that model and the best performing model, w_i is the Akaike weight describing the likelihood that model is the best model out of all the models with $\Delta_i < 2$, and only models with $\Delta_i < 2$ are shown.

Variables included in Model	Δ_i	w_i	R^2_{adj}
Kiamichi River - Diptera			
Re	0.000	0.187	0.043
% Cobble	0.527	0.144	0.027
Re, % Cobble	1.234	0.101	0.033
% Silt	1.293	0.098	0.003
Re, Depth	1.517	0.088	0.025
Re, % Silt	1.663	0.082	0.020
Re, % Sand	1.757	0.078	0.017
Depth, % Cobble	1.800	0.076	0.016
Re, % Gravel	1.875	0.074	0.014
Re, Substrate Roughness	1.947	0.071	0.011
Kiamichi River - Trichoptera			
Slope, % Cobble, % Silt	0.000	0.303	0.706
Slope, % Cobble, % Sand, % Silt	0.893	0.194	0.705
Slope, Depth, % Cobble, % Silt	1.536	0.141	0.700
Slope, % Cobble, % Gravel, % Silt	1.817	0.122	0.697
Slope, % Cobble, % Silt, Substrate Roughness	1.840	0.121	0.696
Slope, % Cobble, % Sand, % Silt, Substrate Roughness	1.853	0.120	0.704
Little River - Diptera			
Re., % Cobble, % Gravel	0.000	0.256	0.390
Re., % Sand	0.055	0.249	0.375
Re., % Boulder, % Sand	1.129	0.145	0.372
Re., % Cobble, % Sand	1.449	0.124	0.367
Re., Depth, % Cobble, % Gravel	1.499	0.121	0.380
Re., Depth, % Sand	1.772	0.105	0.362
Little River - Trichoptera			
Fr, Slope	0.000	0.579	0.425
Fr, Slope, % Sand	0.637	0.421	0.429

Table 3. Summarized results from contrasts testing for biodiversity effects. "Liberal" contrasts test the null hypothesis that polyculture performance can be predicted additively from performance of its monocultures. "Conservative" contrasts test the null hypothesis that polyculture performance is the same as its monoculture with the strongest effect on gravel erosion (see methods). Cohen's *d* estimates effect size (see methods), bold values highlight $d > 0.8$ and $P < 0.05$, "High" and "Low" refer to density treatments, "Act" refers to *Actinonaias ligamentina*, "Amb" refers to *Amblema plicata*, "Quad" refers to *Quadrula pustulosa*, and "3 spp." refers to the 3-species polyculture.

Liberal				***Conservative***			
Contrast	***t***	***d***	***P***	**Contrast**	***t***	***d***	***P***
Algae production rates							
Act + Amb	–0.264	–0.095	0.794	Act + Amb	0.127	0.046	0.900
Act + Quad	1.100	0.395	0.280	Act + Quad	0.354	0.127	0.726
Amb + Quad	–0.844	–0.303	0.405	Amb + Quad	–1.539	–0.553	0.134
3 spp.	0.857	0.308	0.398	3 spp.	–0.524	–0.190	0.604
Aquatic insect emergence rates							
Act + Amb	2.761	**0.992**	**0.010**	Act + Amb	2.825	**1.014**	**0.008**
Act + Quad	1.769	0.635	0.087	Act + Quad	1.355	0.487	0.185
Amb + Quad	2.349	**0.844**	**0.025**	Amb + Quad	1.200	0.431	0.239
3 spp.	1.126	0.404	0.269	3 spp.	0.381	0.137	0.706

Table 4. Summary of the models using algae production rates and algae NMDS axis 1 to predict the emergence rates of aquatic insects for each mesocosm (all models include treatment block as a covariate). Δ_i is the AIC difference between that model and the best performing model, and w_i is the Akaike weight describing the likelihood that model is the best model out of all models.

Model	Δ_i	w_i	R^2_{adj}
Full Dataset			
NMDS Axis 1 (quadratic)	0.000	0.988	0.571
NMDS Axis 1 (quadratic) + Algae Production Rate (linear)	8.937	0.011	
NMDS Axis 1 (linear)	16.410	0.000	
Algae Production Rate (linear)	19.365	0.000	
NMDS Axis 1 (quadratic) + Algae Production Rate (quadratic)	19.838	0.000	
Algae Production Rate (quadratic)	19.944	0.000	
NMDS Axis 1 (linear) + Algae Production Rate (quadratic)	22.253	0.000	
NMDS Axis 1 (linear) + Algae Production Rate (linear)	23.393	0.000	
Dataset excluding possible influential data point on NMDS axis 1			
NMDS Axis 1 (quadratic)	0.000	0.724	0.570
NMDS Axis 1 (linear)	2.052	0.259	0.448
NMDS Axis 1 (quadratic) + Algae Production Rate (linear)	8.922	0.008	
NMDS Axis 1 (linear) + Algae Production Rate (linear)	9.051	0.008	
Algae Production Rate – Linear	14.472	0.001	
NMDS Axis 1 (linear) + Algae Production Rate (quadratic)	15.763	0.000	
Algae Production Rate (quadratic)	18.984	0.000	
NMDS Axis 1 (quadratic) + Algae Production Rate (quadratic)	19.855	0.000	

FIGURES

Figure Legends

Figure 1. Photograph of the mesocosms used in chapter 3, showing the unglazed clay tile and three silica disks used for sampling algae, and the trap used for sampling emergent aquatic insects.

Figure 2. Log-transformed response variables from the mesocosm experiment as a function of mussel species richness. Mussel treatment means and standard errors shown, "Act" refers to *Actinonaias ligamentina*, "Amb" refers to *Amblema plicata*, "Quad" refers to *Quadrula pustulosa*, and "3 spp." refers to the 3-species polyculture. A) Algae Production Rate (mg Chl $a \cdot m^{-2} \cdot d^{-1}$), $y = 0.729 + 0.036x$, $P = 0.029$, $R^2 = 0.120$; B) Aquatic Insect Emergence Rate ($mg \cdot m^{-2} \cdot d^{-1}$), $y = 1.930 + 0.160x$, $P < 0.001$, $R^2 = 0.277$; C) mean spider standing crop biomass (mg), $y = 1.217 + 0.039x$, $P = 0.010$, $R^2 = 0.161$.

Figure 3. Algae NMDS axes plots from mesocosm experiment: axis 1 has R^2 of 0.61 and is positively correlated with diatoms and negatively correlated with non-diatom taxa (primarily green algae), axis 2 has R^2 of 0.19 and separates samples at a finer scale within the diatom/non-diatom groups. A) axes means and standard errors for mussel treatments, "Act" refers to *Actinonaias ligamentina*, "Amb" refers to *Amblema plicata*, "Quad" refers to *Quadrula pustulosa*, and "3 spp." refers to the 3-species polyculture. B) filled contour plot with log-transformed aquatic insect emergence rate ($mg \cdot m^{-2} \cdot d^{-1}$) shown as the color spectrum on top of the NMDS axes plot. The black line indicates

point of separation between data and one possible influential datum that is located at the positive end of NMDS axis 1.

Figure 4. Summary of analyses investigating mechanisms for mussel effects on algae and spiders. A) linear regression using Mussel Derived Nitrogen Index of PMB enrichment relative to available mussel pool to predict algae production rates in week 8; $y = 0.157 + 0.924x$, $p < 0.001$, $R^2 = 0.49$. B) linear regression using Mussel Derived Nitrogen Index of PMB sampled in week 8 to predict NMDS axis 1, $y = -0.796 + 3.288x$, $p = 0.004$, $R^2 = 0.22$. C) log-transformed spider standing crop biomass (mg) as a function of log-transformed aquatic insect emergence rate ($mg \cdot m^{-2} \cdot d^{-1}$), $y = 1.011 + 0.121x$, $P = 0.004$, $R^2 = 0.20$. In A and B, points with open circles represent mesocosms containing *Actinonaias ligamentina,* filled circles are those without.

Figure 5. Standardized aquatic insect emergence rates ($mg \cdot m^{-2} \cdot d^{-1}$, medians ± SE) and mussel species richness for study sites on the Little and Kiamichi Rivers. Mixed-effect regression with random river effect is shown as separate lines for each river (overall model: $y = -1.70 + 0.118x$, $P = 0.006$, $R^2_{adj} = 0.69$).

Figure 1.

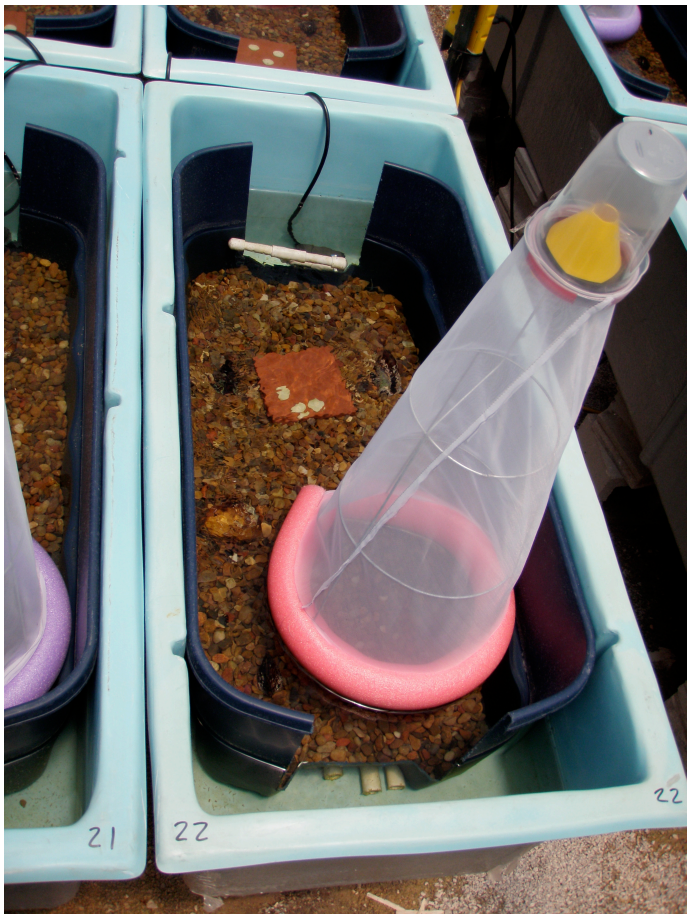


Figure 2.

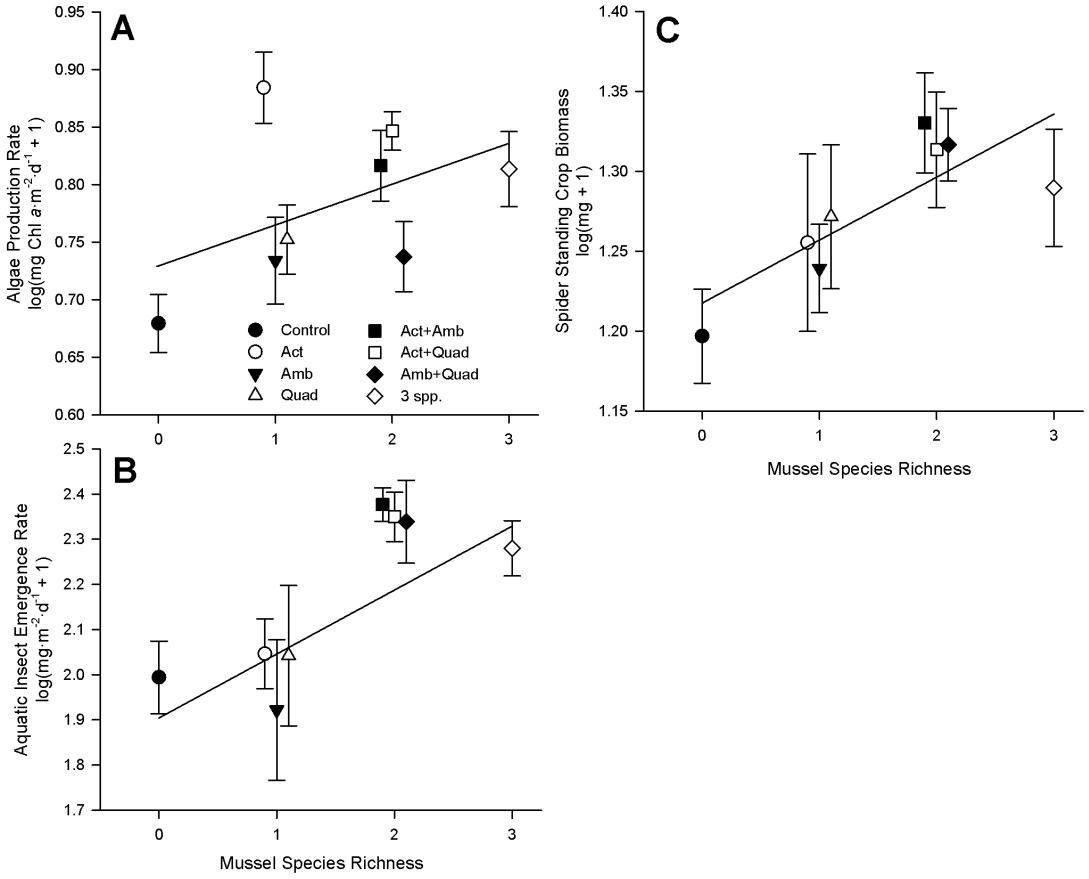

A
B
C
Algae Production Rate
log(mg Chl a·m-2·d-1 + 1)
Aquatic Insect Emergence Rate
log(mg·m-2·d-1 + 1)
Spider Standing Crop Biomass
log(mg + 1)
Control
Act
Amb
Quad
Act+Amb
Act+Quad
Amb+Quad
3 spp.
Mussel Species Richness
Mussel Species Richness

Figure 3.

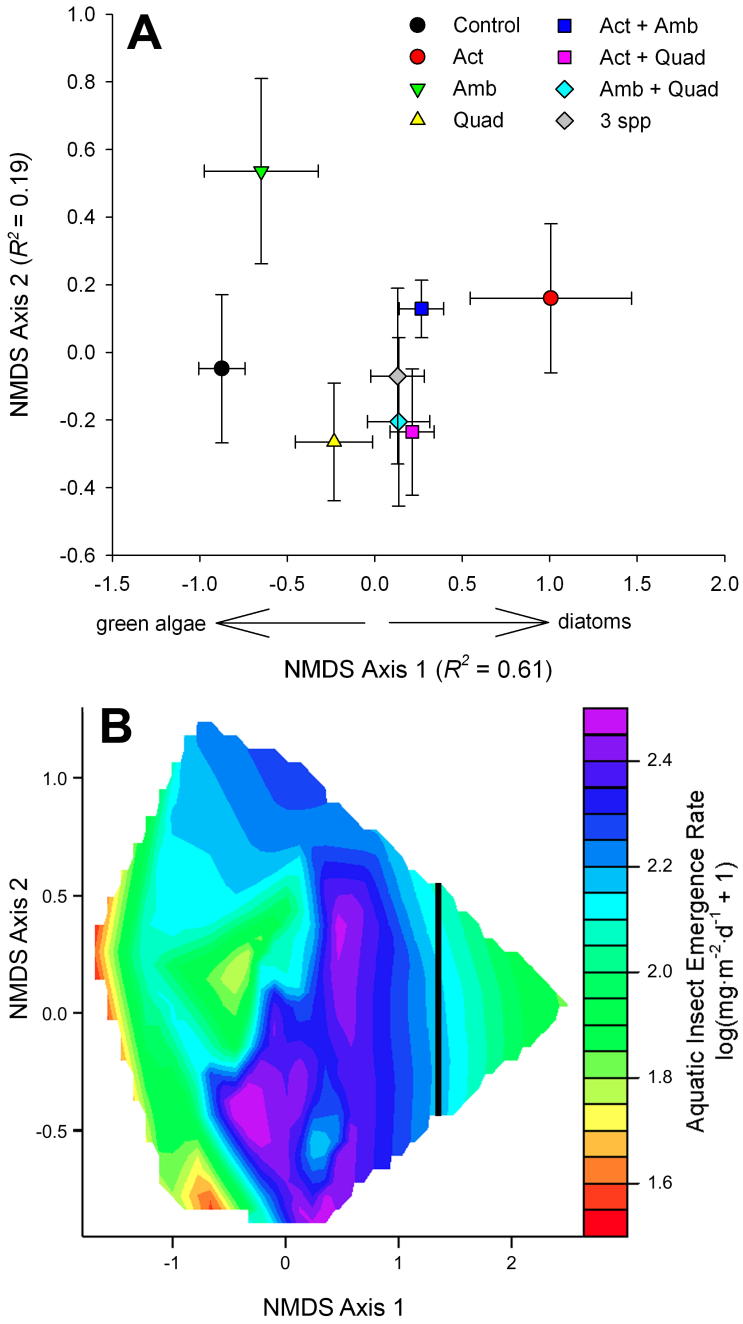

A
Control
Act
Amb
Quad
Act + Amb
Act + Quad
Amb + Quad
3 spp
NMDS Axis 2 (R^2 = 0.19)
green algae
diatoms
NMDS Axis 1 (R^2 = 0.61)
B
NMDS Axis 2
NMDS Axis 1
Aquatic Insect Emergence Rate
log(mg·m^{-2}·d^{-1} + 1)

Figure 4.

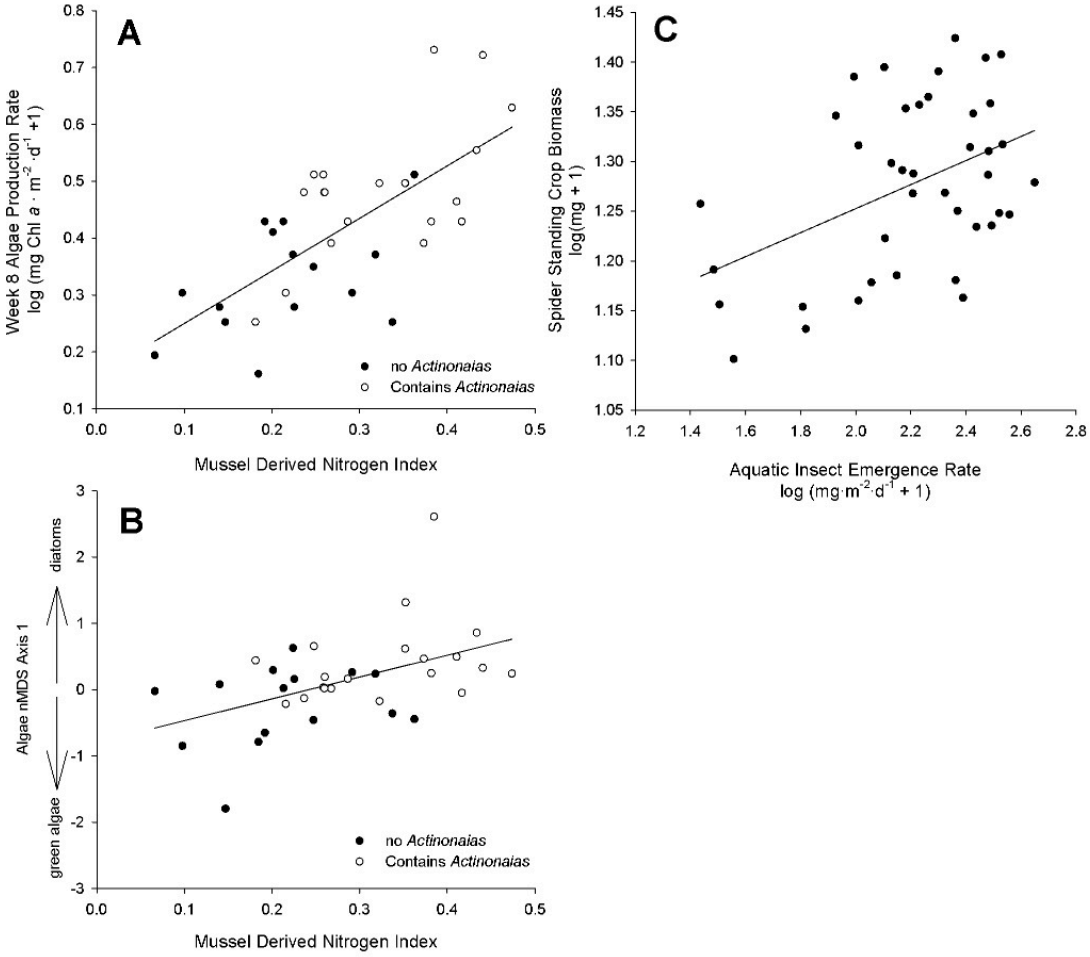
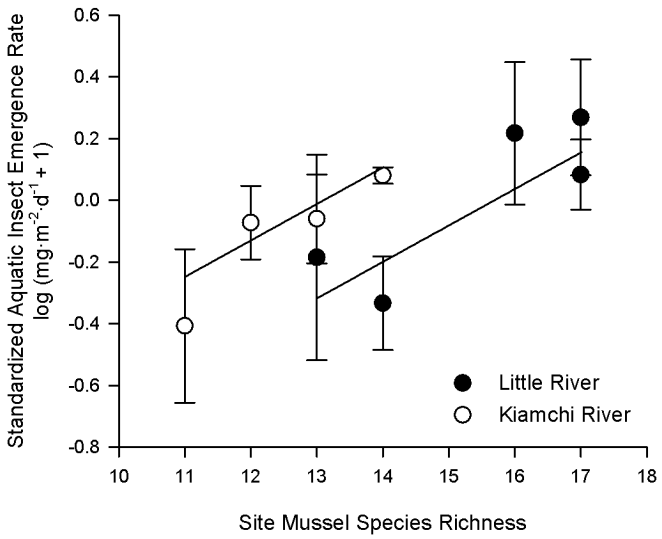

A
Week 8 Algae Production Rate
log (mg Chl a · m-2 ·d-1 +1)
Mussel Derived Nitrogen Index
no Actinonaias
Contains Actinonaias
B
Algae nMDS Axis 1
diatoms
green algae
Mussel Derived Nitrogen Index
no Actinonaias
Contains Actinonaias
C
Spider Standing Crop Biomass
log(mg + 1)
Aquatic Insect Emergence Rate
log (mg·m-2·d-1 + 1)

Figure 5.


Standardized Aquatic Insect Emergence Rate
log (mg·m-2·d-1 + 1)
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
10
11
12
13
14
15
16
17
18
Site Mussel Species Richness
Little River
Kiamchi River